DOCTORAL THESIS

Maximising endurance performance in the heat
investigating the effects of hydration, cooling and heat acclimation interventions

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Maximising Endurance Performance in the Heat: Investigating the Effects of Hydration, Cooling and Heat Acclimation Interventions

by

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This is for you Papa, WE did it!!

Abstract

Prolonged exercise in hot environmental conditions places the body under far greater physiological and perceptual strain than when exercising at the same intensity in temperate conditions, resulting in an impaired exercise performance or capacity. The exact mechanisms that limit exercise performance are a complex interplay between the physiological and the behavioural systems, likely governed by a rising core temperature (hyperthermia). The three main interventions; hydration, cooling and heat acclimation (HA) have been investigated in an attempt to offset the reduction in exercise performance and capacity observed. The current thesis examined the effect of perceived hydration status (Chapter 3) and pre and per-cooling in isolation or in combination (Chapter 4) on the physiological and perceptual responses during a preload and 15 min time-trial (TT) performance in the heat (35 ºC to 40 °C, 50% rh). In Chapter 5, Chapter 6 and Chapter 7 a daily 60 min isothermic HA protocol was investigated on the physiological, perceptual, cognitive function and lipopolysaccharide responses. To further compare whether there was a time course effect over short-term (five days) and medium-term (ten days) upon these responses. Perceived euhydration status when hypohydrated had no effect on 15 min TT performance in moderately trained individuals, when the physiological and perceptual consequences of hypohydration existed. Neither external pre- nor per-cooling in isolation or in combination improved 15 min preloaded TT performance in the heat in

highly trained athletes (Chapter 4). Pre-cooling via whole-body cold water immersion (CWI); 22 °C) for 30 min was successful at lowering overall strain for a limited period during the preload; however, by the time participants commenced the TT, the majority of these benefits had worn off, with combined external per-cooling not providing any additional benefit. A short-term (five days) 60 min daily isothermic (target rectal temperature ~38.5 °C) HA protocol provided a sufficient thermal impulse to elicit the necessary thermal adaptations to reduce physiological and perceptual strain when exercising in the heat, without inducing an endotoxin response with medium-term (ten days) offering a more complete adaptation to some systems and offering protection to working memory function.

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Publications

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Table of Contents

List of Figures

List of Tables

List of Abbreviations

%	Percentage
ºC	Degrees Celsius
ACSM	American College of Sports Medicine
ANOVA	Analysis of variance
$b.min^{-1}$	Beats per minute
BM	Body mass
CC	Cooling collar
CF	Cognitive function
h	Hour
HA	Heat acclimation
Hb	Haemoglobin
Hct	Haematocrit
HR	Heart rate
HST	Heat stress test
HYP	Hypohydration
kg	Kilogram
kJ	Kilojoule
km	Kilometre
L	Litre
m	Meter
min	Minute

mL	Millilitre
ms	Millisecond
MTHA	Medium-term heat acclimation
PPO	Peak power output
PV	Plasma volume
rh	Relative humidity
RPE	Ratings of perceived exertion
s	Second
SD	Standard deviation
STHA	Short-term heat acclimation
TC	Thermal comfort
T_{re}	Rectal temperature
TS	Thermal sensation
TTE	Time to exhaustion
TT	Time trial
$\dot{V}O_{2max}$	Maximal oxygen uptake
$\dot{V}O_{2peak}$	Peak oxygen uptake
uL	Microlitres
USG	Urine specific gravity
WBGT	Wet-bulb globe temperature
W_{max}	Maximal power output

Chapter 1. **Introduction**

A number of major international sporting events take place during the summer months in the northern hemisphere. The thermal stress experienced in such events imposes an additional physiological and perceptual strain on athletes that can impair exercise performance (Maughan and Shirreffs, 2004). One of the early observations of the detrimental effects from exercising in the heat was observed during the marathon race at the Olympic Games in 1904, which was held in St. Louis where ambient temperatures were recorded to exceed 30 °C. The British-born marathon runner, Thomas Hicks, finished first but had to be carried across the finish line by his support team. Hicks was severely dehydrated and had a slow overall marathon performance time compared to previous marathon times (Marino, 2004; Peiser and Reilly, 2004). Similar observations were made during the marathon race at the London Olympic Games in 1908. Dorando Pietri, was in the lead from mile 25 but collapsed upon entering the stadium, which was claimed to be due to heat exhaustion and a lack of preparation for these environmental conditions (Wood and Maughan, 1999).

For decades now, it has been well-documented that exercise capacity and performance are progressively impaired with increasingly hot environmental conditions (Galloway and Maughan, 1997; Parkin *et al.*, 1999; Tatterson *et al.*, 2000). Galloway and Maughan (1997) demonstrated that as ambient temperature increased, the ability to continue exercising in a laboratory-controlled setting decreased (**Error! Reference source not found.**) and a number of investigations have observed similar response

within real-world race settings. For example, Ely, Cheuvront and Montain (2007) reported that marathon times in both sexes were 4.5% to 5.4% slower than the course record as Wet-bulb globe temperature (WBGT) increased from the ranges of 5.1 ºC to 10 ºC up to the range of 20.1 ºC and 25 °C.

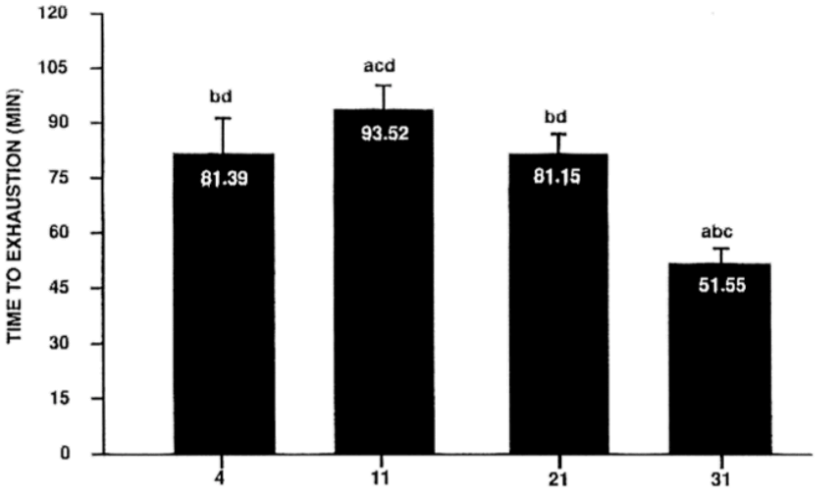

Figure 1.1. Time to exhaustion under four different ambient temperatures. Data are presented as mean and standard error of mean. Letters a, b, c, d indicate a significant difference ($P < 0.05$) from corresponding values at 4 ºC, 11 ºC, 21 ºC and 31 ºC, respectively. [Reproduced from Galloway and Maughan (1997), with permission].

With clear evidence demonstrating an impaired exercise capacity and performance when exposed to hot environmental temperatures, understanding the mechanisms behind these causes of hyperthermia induced decrements has been a topic of interest (Nybo, Rasmussen and Sawka, 2014; Guy *et al.*, 2015). Despite a substantial body of research, the precise mechanism/s to limit exercise performance in a hot environment remain to be fully elucidated and, ultimately, is likely a complex interplay of multiple factors (Abbiss and Laursen, 2007). In the following chapter of this thesis, the proposed physiological and perceptual mechanisms will

be discussed, with attention drawn to the effect exercise-induced hyperthermia has on exercise capacity and performance.

The human body has a tightly regulated internal temperature that is maintained at ~37 °C regulated by the preoptic area of the hypothalamus (known as the thermoregulatory centre of the brain) (Natarajan, Northrop and Yamamoto, 2015). Normal cyclical fluctuations occur in internal body temperature during the day (circadian rhythm; where the highest readings are typically seen in the evening with the lowest in the mornings), in females (menstrual cycle) (Garcia *et al.*, 2006) and throughout life-time (aging) (Tansey and Johnson, 2015). Regardless of these cyclical variations, heat transfers between the body and the environment through four mechanisms of heat exchange; conduction, convection, radiation and evaporation. When ambient air temperature is equal to or greater than skin temperature, the gradient for heat exchange is reversed and the body gains heat via radiation and convection (Baker, 2017). Heat exchange by evaporation of sweat becomes the primary mechanism of heat removal during exercise and can account for ~80% of heat loss when humidity is low. However, evaporative heat loss is only effective when liquid water changes into water vapour, which is determined by the combination of the surface area of the skin exposed to the environment, air movement (convection), the ambient air temperature and water vapour gradient between the skin surface and the air (Gagge and Nishi, 2011). The combination of metabolic heat production from exercise and high environmental air temperatures, imposes an additional challenge to the

thermoregulatory system, where body temperature is unable to be maintained and hyperthermia develops. Hyperthermia will be defined throughout this thesis as an increase in core body temperature above a set range, which is ~37 °C during rest and ~38 °C during moderate-intensity exercise (Nybo, 2008).

Exercise-induced hyperthermia has been hypothesised to be one of the limiting factors to exercise capacity and aerobic performance (Marino, 2002). The elevation in core temperature response during exercise is dependent upon a number of variables including; the mode, intensity and duration of exercise, and the thermal environment that exercise is performed in (Burton, Stokes and Hall, 2004). At the onset of exercise, metabolism increases rapidly to meet the energetic demands of skeletal muscle contraction (González-Alonso *et al.*, 2000; Bangsbo *et al.*, 2001; Sawka *et al.*, 2011; Nybo, Rasmussen and Sawka, 2014). Due to the inefficiency of metabolic reactions required to provide energy to the working muscle and the delayed thermoregulatory effector responses to dissipate heat, there is an initial rise in muscle temperature within the first few contractions (1 s - 3 s) and a rise in core body temperature typically occurs within the first 3 min of exercise (Gleeson, 1998; González-Alonso *et al.*, 2000; Bangsbo *et al.*, 2001). With mechanical efficiency poor and dependant on rate of oxygen consumption ($\dot{V}O_2$), only ~20% to 25% of this chemical energy is transferred into mechanical energy (Moseley *et al.*, 2004) and therefore, up to ~80% of this metabolic heat is converted into thermal energy (heat) (Cramer and Jay, 2016). As exercise progresses

mechanical efficiency declines as there is a greater shift to aerobic energy contribution, due to the metabolic cost of adenosine triphosphate (ATP) production increasing during the latter stages of exercise (Krustrup *et al.*, 2003).

In an attempt to offset exercise-induced hyperthermia, and therefore, minimise the detrimental effects to exercise performance in the heat, a number of interventions have been investigated. The three key interventions proposed to alleviate thermal strain and explored within this thesis are hydration, cooling, and heat acclimation (HA). Throughout this thesis heat stress refers to the net heat load from a combination of metabolic heat production and environmental factors (i.e. ambient air temperature and humidity) and thermal strain is defined as the overall physiological responses to occur from heat stress. Physiological strain is defined within this thesis as changes in two physiological systems, which are increases in rectal temperature and in heart rate. This thesis contains seven main chapters (Chapters 2 - 8); the chapters are outlined below:

- **Chapter 2** presents the review of the literature that highlights the detrimental effects of hot environmental temperatures on of exercise capacity and performance, followed by a discussion of the proposed mechanisms to explain why this may occur. The review will then focus on hydration, cooling and HA interventions proposed to offset the increased physiological and perceptual strain that limit exercise performance in the heat.

- **Chapter 3** investigated the influence of perception and expectation of hydration status on exercise capacity and performance in the heat in moderately trained individuals.
- **Chapter 4** explored the effects of external pre- and per-cooling via the application of neck cooling collar used in isolation and in combination with cold water immersion on exercise capacity and on 15 min cycling time trial performance in the heat, in highly trained athletes.
- **Chapter 5** investigated the effects of a 60 min daily isothermic HA protocol (target rectal temperature ~38.5 °C) on the physiological and perceptual responses to exercise heat stress. This chapter investigated whether there was a time course effect to these adaptations over a short-term (five days) and medium-term (ten days) time course.
- **Chapter 6** presents data collected during experimental Chapter 5 and investigated the effects of acute heat exposure on cognitive function and whether a short-term and medium-term 60 min daily isothermic HA protocol had any protective benefit on cognitive function.
- **Chapter 7** presents data collected during experimental Chapter 5 and investigated whether a 60 min daily isothermic HA protocol would induce an endotoxin response to exercising in the heat, in addition to determining whether there was a time-course effect on this response if so.
- The final chapter of this thesis **(Chapter 8)** discusses and summaries the results from the five preceding Chapters and proposes questions that future research may look to explore.

Chapter 2. **Literature Review**

2.1. Methodology for review of literature

The publications included in this literature review were searched using electronic databases including; PubMed, Google Scholar and Research Gate. Keywords were used during the search process which were; ‘acclimation’, 'adaptation' ‘capacity’, ‘cognitive’, ‘cold water immersion’, ‘controlled hyperthermia’, ‘cooling’, ‘dehydration’, ‘endotoxin’, ‘exercise’, ‘heat’, ‘hot’, ‘hydration’, ‘hypohydration’, ‘isothermic’, ‘lipopolysaccharide’, ‘neck cooling’, ‘performance’, ‘per-cooling’, ‘pre-cooling’, ‘temperature’, ‘thermoregulation’. Articles chosen were primarily focused upon human studies with a few included on animal studies, published in English-language peer-reviewed journals.

2.2. The environmental parameters of heat stress

One of the greatest threats to temperature regulation is the thermal environment, which is a combination of four environmental parameters that influence body temperature when exercise is combined with exposure to heat stress (Maughan, 2010; Otani *et al.*, 2016, 2018). The four main parameters are:

1) Ambient air temperature
2) Relative humidity
3) Air velocity
4) Solar radiation

2.2.1. Ambient air temperature

Ambient air temperature is the temperature of the air usually measured on a scale in either degree Celsius (°C), degrees Fahrenheit (°F) or Kelvin (K) (Howells, 2015). For the purpose of this thesis, degree Celsius will be referred to, whereby 0 ºC represents the freezing point of water and 100 ºC represents boiling point of water.

2.2.2. Relative Humidity

Relative humidity is the percentage (%) of water vapour in the air. One hundred percent relative humidity (rh) means that the air is 100% saturated with water vapour and therefore, is unable to hold any more water. During exercise in the heat, heat loss by the evaporation of sweat from the skin surface becomes the primary mechanism to regulate body temperature and can account for 80% of heat loss when humidity is low (Gisolfi and Mora, 2000); however, when humidity is high the ability to dissipate heat by evaporation becomes limited (Maughan, Otani and Watson, 2012; Che Muhamed *et al.*, 2016).

The restricted heat loss capabilities caused by high humidity can result in an impaired exercise performance. Maughan, Otani and Watson (2012) investigated the effects of four different relative humidity conditions (24%, 40%, 60% and 80% rh) on cycling time to exhaustion (TTE) at 70% maximal oxygen uptake ($\dot{V}O_{2max}$) in the heat (30.2 ± 0.2 ºC). The authors reported TTE to be progressively impaired at 60% (14.5 ± 8.6 min) and

80% rh (22.1 ± 11.0 min) when compared to cycling in 24% rh (68 ± 19 min). More recently, Che Muhamed *et al.* (2016) reported, that an individual's capacity to continue exercising was reduced at 61% rh and 71% rh, compared to when exercising at 23% and 53% rh and that the increasing relative humidity was associated with a linear increase in a thermoregulatory and circulatory strain (to be discussed in section 2.4.1).

2.2.3. Air velocity

Air velocity measures the movement of air and pressure of air across a surface (e.g. the body). The absence of adequate airflow will influence the rate at which heat is lost by both convection and evaporation (Saunders *et al.*, 2005). In the presence of sufficient airflow, thermoregulatory and cardiovascular strain are lower compared to with no or limited airflow (Shaffrath and Adams, 1984; Adams *et al.*, 1992). As well as the physiological state of an individual being highly influenced by airflow, ratings of perceived exertion (RPE) and perceived thermal strain have also been reported to be lower as a result of sufficient airflow during exercise in the heat (Saunders *et al.*, 2005; Otani *et al.*, 2016). Saunders *et al.* (2005) was one of the first to systematically examine the effects of gradual variations in facing air velocities (which were based upon varying degrees of what might be expected during cycling outdoors) - 0 $km \cdot h^{-1}$, 10 $km \cdot h^{-1}$, 33 $km \cdot h^{-1}$, and 50 $km \cdot h^{-1}$. On four separate occasions, participants completed a fixed intensity exercise bout lasting 120 min in the heat (33 °C, 59% rh). During 0 $km \cdot h^{-1}$, none of the participants were able to

complete the trial but all participants completed the exercise bout when the wind speed was 50 $km \cdot h^{-1}$. This study reported that as airflow decreased there was an increase in thermoregulatory and cardiovascular strain and a decrease in exercise tolerance. These findings and other recent evidence (Teunissen *et al.*, 2013) demonstrated that prolonged exercise is impaired in still wind conditions whereas exercise performed in higher air velocity environmental conditions is substantially improved (Otani *et al.*, 2016). The presence of adequate air flow allows for a greater rate of heat loss by both convection and evaporation, leading to a reduced amount of heat being stored (Saunders *et al.*, 2005).

2.2.4. Solar radiation

Solar radiation is the radiant energy that is emitted by the sun and contains a considerable amount of ultraviolet radiation (UV). Solar radiation is essential for life and is a vital source of heat, which increases air temperature (Stahl and Sies, 2001). Exercise performance has been reported to progressively deteriorate as solar radiation increases, likely due to the influence that solar radiation has on the thermoregulatory system, increasing both sweat rate (Nielsen, Kassow and Aschengreen, 1988) and skin temperature (Nielsen, Kassow and Aschengreen, 1988; Otani *et al.*, 2016). A limitation to studies conducted in a climate chamber, is that certain environmental variables such as solar radiation are rarely considered and therefore, it is important to review the literature that has

investigated this in an outdoor setting or in environmental chambers that have ceiling-mounted solar simulators.

In an earlier study, Nielsen, Kassow and Aschengreen (1988) investigated the physiological effects of heat load when exercising during sunny days between July and September in Denmark. The authors observed a decrease in skin temperature, heart rate (HR) and oxygen uptake when participants changed from cycling in direct sun exposure to shade where solar radiation was decreased. More recently, Otani *et al.* (2016) reported that a cycling TTE in a hot environmental temperature (30 ºC) was shortest (23 ± 4 min) during the highest solar radiation (800 watts per square meter ($W \cdot m^2$)) trial, compared to all other trials (500 $W \cdot m^2$: 30 ± 7 min; 250 $W \cdot m^2$: 42 ± 10 min; 0 $W \cdot m^2$: 46 ± 10 min, all $P < 0.001$). While no differences were reported between trials in rectal temperature, sweat losses, HR and skin blood flow, the increasing solar radiation was associated with a progressive increase in skin temperature, narrowing the thermal gradient between core and skin.

2.2.5. Heat stress indices

The four environmental parameters of heat stress indices can be measured independently but doing so fails to represent the true environmental heat stress. There are a number of indices used to assess and evaluate the environmental conditions to ensure athletes are safe to perform in such environments. These models include, universal thermal climate index (UTCI), wet-bulb globe temperature (WBGT), wet-bulb dry temperature

(WBDT) and tropical summer index (TSP). In brief, the UTCI is used to measure the heat stress in outdoor environments, calculating temperature, average radiation temperature, air velocity and humidity (Blazejczyk *et al.*, 2012). WBGT is the most widely used index of heat stress and was first investigated by the US navy in the 1950s in order to minimise heat related illness during military training camps (Yaglou and Minard, 1957; Budd, 2008). WBGT combines readings from two to three thermometers to measure the combined effect of ambient air temperature, radiant heat and air velocity.

2.2.6. Heat stress summary

The interactions between the aforementioned environmental parameters are the cause of heat stress rather than ambient air temperature alone. Consequently, the input of these four environmental parameters affect the physiological and behavioural responses during exercise in a thermally challenging environment. Laboratory designed studies often fail to meet the ecological validity of thermal stress when compared to data collected during field studies. The environmental parameters investigated in isolation and in combination can impair exercise capacity and exercise performance, this will be reviewed in the upcoming section.

2.3. The effects of environmental temperature on exercise

2.3.1. The effects of environmental temperature in field studies

Environmental temperatures have been well-documented to influence an individual's ability to perform, with increasingly hot and humid environmental temperatures imposing a greater physiological and perceptual strain. The elevated strain can result in an impaired exercise performance (Ely *et al.*, 2007; El Helou *et al.*, 2012; Guy *et al.*, 2015), but not all sporting performances are impaired by increasingly hot environmental conditions. In one study, data were obtained from the IAAF track and field performances between 1999 to 2011 and subsequently analysed by Guy *et al.* (2015). Environmental conditions were categorized into temperate (<25 °C) and hot (>25 °C), with eight running events included in the analysis (100 m, 200 m, 400 m, 800 m, 1500 m, 5000 m, 10,000 m & the marathon).

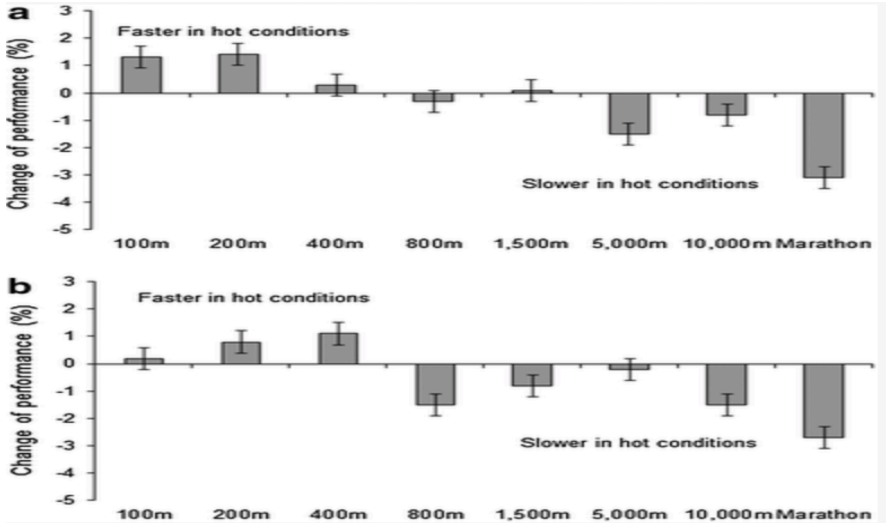

Figure 2.1. Comparative mean ± 95 % CL percentage change of performance in temperate (<25 °C) vs hot (≥25 °C) conditions from International Association of Athletics Federation (IAAF) World Championship track events from 1999–2011 for *a* males and *b* females. Positive percentage indicates faster performance, and negative percentage indicates slower performance in hot conditions. [Reproduced from Guy *et al*. (2015), with permission].

Endurance events (>5000 m) performed in temperate conditions produced faster times than when exposed to hot environmental conditions. Contrary to these findings, in sprinting events (<200 m) athletes performed faster in hot than temperate environmental conditions (Figure 2.1). A likely explanation for the improved sprint performance in the heat is the increase in muscle temperature (Girard, Brocherie and Bishop, 2015) which accelerates muscle fibre conduction velocity and upregulates the enzymatic processes (Gray *et al.*, 2006) resulting in an increase in power output.

The findings from Guy *et al.* (2015), illustrated above in Figure 2.1. on the slowing of marathon performance times in the heat (>25 °C) in both males and females had previously been reported by Ely *et al.* (2007). Marathon results and weather data were obtained from Boston, New York, Twin Cities, Grandma's, Richmond, Hartford, and Vancouver races. These events were categorized on environmental heat stress (5 ºC to 25 °C), and were broken down into quartiles based on WBGT. In general, marathon performance time progressively slowed as WBGT increased from 5 ºC to 25 °C; however, faster runners demonstrated a smaller impairment in marathon performance time (~2% to 3%) compared to the slower runners (~10%) (Figure 2.2). After further analysis, Ely *et al.* (2008) reported that slower runners were observed to decrease their running pace as the race progressed, while faster runners, adjusted their running pace from the start of the race, maintaining an even running velocity over the 42.2 km distance.

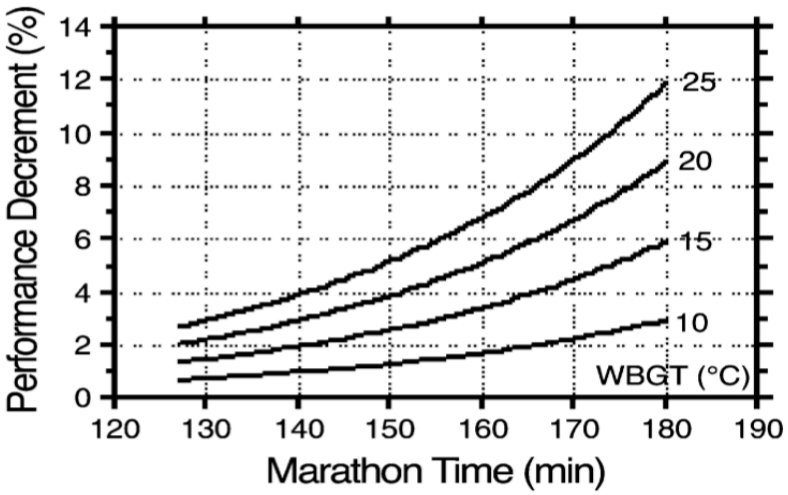

Figure 2.2. Nomogram examining the potential performance decrement (y-axis) based on projected marathon finishing times (x-axis) with increasing WBGT. [Reproduced from Ely et al. (2007), with permission].

As well as running ability, a person's sex also influences running performance, with women better able to preserve running performance at slightly higher environmental temperatures than men (9.9 °C vs. 6 °C; Ely *et al.*, 2008; El Helou *et al.*, 2012). Cooler environmental temperatures (5 ºC to 10 °C) have been associated with the fastest marathon times, where running velcoity is better maintained during the marathon race compared to warmer conditions (Montain, Ely and Cheuvront, 2007; Ely *et al.*, 2008).

Recent evidence from El Helou *et al.* (2012), who obtained marathon performance data from three European (Paris, London & Berlin) and three American races (Boston, Chicago & New York) between the years of 2001 - 2010, which included a total of 1,791,972 runners, highlighted that air temperature was the most influential environmental parameter on

marathon performance in both sexes, with humidity the second most important parameter. The optimal air temperature for maximal running velocity for women was reported to be 9.9 °C, and an increase of 1 °C from this temperature resulted in a loss of running velocity of 0.03%. This optimal temperature was reported to be lower in men, where maximal running velocity was observed during environmental conditions of 3.81 °C. Not only does increasing environmental temperatures impair performance time but also the number of individual's finishing the race decreases (El Helou *et al.*, 2012). For example, only ~54% of runners who started the Olympic marathon race completed the event when ambient temperatures exceed 25 °C compared to ~79% of runners who finished when ambient temperatures were lower than 25 °C (Martin and Gynn, 2000).

2.3.2. The effects of environmental temperature in laboratory settings

Time to exhaustion (TTE) and time trials (TT) are two tests commonly used within the laboratory setting to measure an exercise capacity and to evaluate exercise performance, respectively (Laursen *et al.*, 2007). During a TTE test, participants perform the exercise bout at a set fixed sub-maximal intensity (i.e. % of their maximal aerobic capacity ($\dot{V}O_{2max}$)) until they voluntarily terminate at exhaustion or are instructed to stop when attaining a prescribed (ethically-determined) core temperature threshold or another criteria of termination (e.g. percentage of max HR) (*this varies*

between Universities). An exercise capacity test is an open-loop design, that allows for the opportunity to obtain physiological and perceptual data at comparable intensities and to compare between intervention or treatment groups (Currell and Jeukendrup, 2008). In contrast, a TT is a closed-loop design test, where the individual completes a known set distance or amount of work in the shortest time possible (Padilla *et al.*, 2000). TTE tests compared to TT tests, have a higher level of variability and are potentially less ecologically valid, as open-ended tests are highly influenced by psychological factors such as motivation and boredom (Jeukendrup *et al.*, 1996; Che Jusoh *et al.*, 2015). Throughout this literature review both exercise protocols will be considered.

Galloway and Maughan (1997) were one of the first to systematically investigate a range of ambient air temperatures (4 ºC, 10.5 ºC, 21 ºC & 30.5 °C) on an individual's capacity to exercise during a TTE cycling protocol (70% $\dot{V}O_{2max}$). The authors observed that the individuals' capacity to exercise was reduced by ~45% when cycling in 30.5 °C environmental conditions (51.6 ± 3.7 min) compared to cycling in 10.5 °C (93.5 ± 6.2 min). Subsequently, Parkin *et al.* (1999) reported TTE to be 65% shorter when cycling in 40 °C (30 ± 3 min) compared to when cycling in 3 °C (85 ± 8 min). More recently, Girard and Racinais (2014) supported the findings of both the preceding studies (Galloway and Maughan, 1997; Parkin *et al.*, 1999), as TTE was reduced by 35 ± 15% when cycling in hot (35 °C, 40% rh) compared to cycling in temperate environmental conditions (22 °C, 30% rh). In the same year, Mitchell *et al.* (2014) reported TTE was halved

when exercising in a hot environment (37 °C) compared to cool environment (10 °C). The findings from these studies provide clear evidence to support that an individual's capacity to exercise is limited when exposed to increasingly hot environmental temperatures (Galloway and Maughan, 1997; Parkin *et al.*, 1999; Girard and Racinais, 2014; Mitchell *et al.*, 2014).

During TT investigations, it has been frequently reported that individuals have a reduced average power output during cycling (Tatterson *et al.*, 2000; Tucker *et al.*, 2004) and a reduced average velocity during running performances (Ely *et al.*, 2007, 2008; El Helou *et al.*, 2012) in hot environmental temperatures. Both Tatterson *et al.* (2000) and Tucker *et al.* (2004) investigated the effects of hot environmental temperatures (32 ºC to 35 °C) compared to moderate-cool environmental temperatures (15 ºC to 23 °C) on cycling performance. Tatterson *et al.* (2000) reported that power output was reduced by 6.5% (345 ± 9 W vs. 323 ± 8 W) during a 30 min TT, with Tucker *et al.* (2004) observing a similar reduction in power output of 6.3% (255 ± 47 W vs. 272 ± 45 W) during a 20 km TT in the heat. More recently, Peiffer and Abbiss (2011) systematically examined the effect of four environmental temperatures (17 ºC, 22 ºC, 27 ºC and 32 °C) on 40 km cycling TT performance. The greatest reduction in power output observed was reported during the hottest environmental temperature investigated, where physiological strain was also the highest. This study and the findings from the previously mentioned (Tatterson *et*

al., 2000; Tucker *et al.*, 2004), support that TT performance is impaired when performed in hot environmental conditions.

2.3.3. The effects of environmental temperature on cognitive function

In the previous section (2.3.1 & 2.3.2) the well-established detrimental effects of hot environmental temperatures on prolonged exercise capacity and performance were reviewed; however, the influence of environmental temperatures on cognitive function still remains equivocal. Cognitive function is an umbrella term that describes all mental tasks that include memory, knowledge, attention, reasoning, problem solving and comprehension and can be categorised into "complex" and "simple" tasks (Ramsey and Kwon, 1992; Lamport *et al.*, 2014). Complex tasks require an individual to have a greater level of attention and effort to complete. These include complex motor coordination and working memory tasks. In comparison, simple tasks require simple motor and memory skills, which include reaction time and memory recall tasks.

To date, the literature lacks consistency with regards to the approaches used to access cognitive function (Tomporowski and Ellis, 1986; Hancock and Vasmatzidis, 2003), and this has resulted in confounding findings (Gaoua, 2010). Some examples of these methodology inconsistencies include severity of heat exposure used (i.e. temperature, humidity, duration), complexity of cognitive performance test (i.e. duration, task type, assessment method) and the participants included (i.e. fitness level,

experience) (Ramsey and Kwon, 1992; Pilcher, Nadler and Busch, 2002; Riniolo and Schmidt, 2006; Gaoua, 2010). A decline in cognitive performance has been observed during environmental heat stress in some (Hocking *et al.,* 2011; Morley *et al.,* 2012; Parker *et al.,* 2013) but not all studies (Bell, Provins and Hiorns, 1964; Nunneley *et al.*, 1979). Task complexity seems to be the primary factor, with the simple tasks mentioned above appearing less vulnerable to heat stress than more complex tasks (Hancock, 1981, 1982; Hancock and Vasmatzidis, 2003).

For the purpose of this review, the focus will be on active hyperthermia relating to cognitive function outcomes. In one study, Hocking *et al.* (2001) investigated the impact of thermal stress on cognitive performance during three conditions; 1) 25 ºC, 65% rh, 2) 35 ºC, 65% rh and 3) 35 ºC, 65% rh with hyperthermia (core temperature ≥38.5 ºC). During the third experimental trial, participants walked for ~40 min at an increase of 5 $km \cdot h^{-1}$ to increase core body temperature before matching the walking speed performed in all other trials (1.8 $km \cdot h^{-1}$) during the cognitive performance testing period. Attention, memory and information processing were tested, while participants' brain electrical activity was measured. Thermal strain was shown to have an impact upon these performance outcomes by showing a decline in working memory as task complexity increased. The authors also found that with increasing thermal strain, there was an increase in steady-state visual evoked potentials (SSVEP). Neural signals that occur in response to a visual stimulus presented at a specific frequencies (Hz), they are delivered at such a high

rate that the response to one stimulus has not died away before the next is received (Regan, 1982). Thermal strain was shown to increase SSVEP but decrease latency in participants, it was suggested by Hocking *et al.* (2001) that cognitive performance was maintained due to a greater utilisation of neural resources, however, it is when this cognitive reserves are overloaded that performance is impaired. Following this, Stubblefield *et al.* (2006) investigated the effects of active hyperthermia on simple and complex cognitive function tasks before and after a heat stress test (HST). During the HST (34.3 ± 2.2 ºC, 50.8 ± 9.9% rh), eight males completed a 15 min warm up (~60% maximum HR) before completing an anaerobic exercise test (10 sets of 10 x 15 s sprints). Complex tasks were reported to be more vulnerable than simple tasks, where a decline of 12.22% in memory function was reported after exercise coinciding with elevated core body temperature (38.8 ± 0.4 ºC). In a meta-analytical review, Pilcher, Nadler and Busch (2002) reported that the length of experimental duration had an influence, where short exposure resulted in worse performance outcomes than compared to longer durations. However, the longer the participant was exposed to environmental temperature prior to task resulted in worse performance outcome. A more recent review by Lambourne and Tomporowski (2010) included acute exercise studies (n = 40) measuring cognitive function prior, during (n = 21) and after an exercise bout (n = 29). The authors reported that during exercise cognitive performance declined, but improved post-exercise. It remains unclear whether it is exercise-induced hyperthermia per se or a combination of

other physiological and perceptual mechanism that compromise cognitive function.

2.3.4. Summary of field, laboratory, and cognitive impairments

It is clear and well-documented that exercise capacity and exercise performance in both field and laboratory studies is impaired in the heat compared to moderate environmental conditions. In performance studies, a reduction in power output or running velocity has been reported, where during exercise capacity tests the ability for an individual to continue exercising at a fixed intensity is reduced with increasing ambient air temperature. Far less known to what extent the impact of environmental heat stress during exercise has on cognitive function, with complex tasks shown to be more vulnerable to thermal strain than simple tasks. Understanding the mechanism/s to why exercise is impaired in these environmental conditions will be proposed in the upcoming section of this review.

2.4. The proposed mechanisms to explain the impaired exercise performance in hot environmental conditions

Hyperthermia induced decrement in exercise performance is defined within this thesis, as the inability to sustain a high-power output during prolonged exercise due to sensations of tiredness (Abbiss and Laursen, 2005). There have been a number of proposed paradigms to explain why performance is impaired in hot environments; however, there is no clear

explanation that currently exists. Exercising in the heat is associated with increased thermoregulatory strain, due to a rise in body temperature from the rate of metabolic heat production and an inability to dissipate heat. Hyperthermia induces a number of homeostatic changes that interact in parallel with changes in the cardiovascular, respiratory, central nervous system and muscular functions that may influence exercise performance in these conditions (Figure 2.3; Nybo, 2008; Nybo, Rasmussen and Sawka, 2014). In the upcoming subsections, the primary focus will be on the physiological and behavioural responses to hyperthermia during prolonged exercise in the heat.

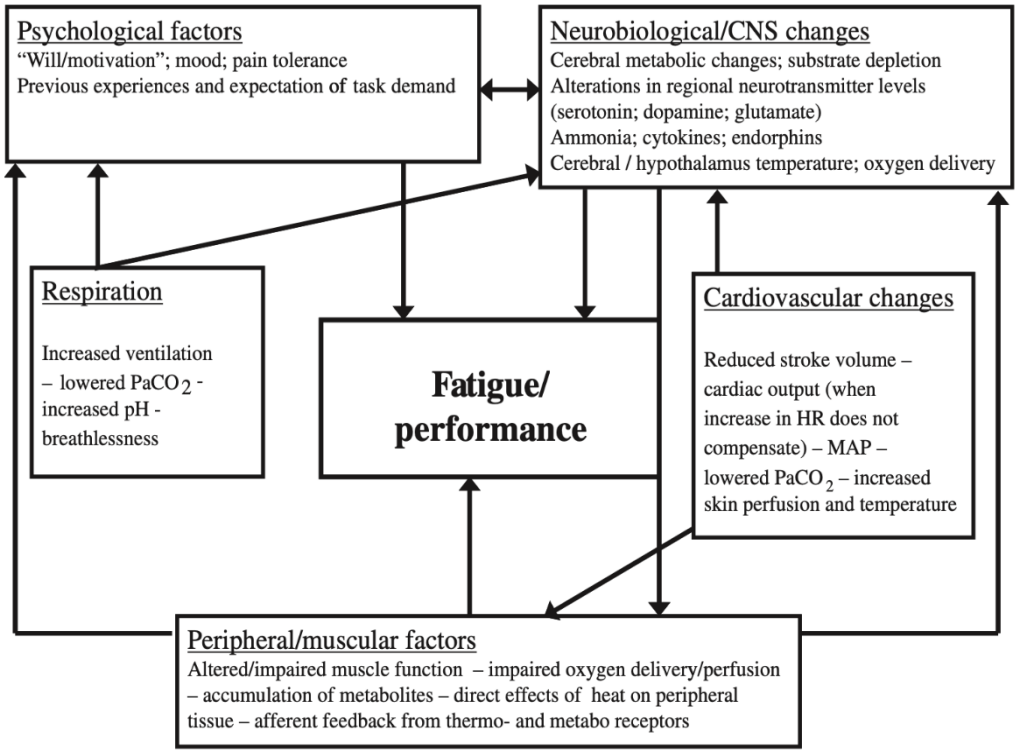

Figure 2.3. Integrative model with the potential cardiovascular, respiratory, central nervous system, and peripheral factors that may influence fatigue during exercise in the heat. [Reproduced from Nybo, Rasmussen and Sawka (2014), with permission].

2.4.1. Increased physiological strain to exercise in the heat

2.4.1.1. Thermoregulatory response to exercise in the heat

The regulation of body temperature occurs through two parallel processes that function in order to achieve body heat balance: behavioural thermoregulation (reviewed later on in section 2.4.2.) and autonomic thermoregulation (Nybo, Rasmussen and Sawka, 2014). When heat exchange mechanisms are insufficient to match heat gain, heat is stored which subsequently leads to a rise in body temperature (Nielsen *et al.*, 1993; Galloway and Maughan, 1997; González-Alonso *et al.*, 1999; Nybo,

Rasmussen and Sawka, 2014). The magnitude at which body temperature is elevated is largely independent of the environmental condition and is proportional to the metabolic heat production (Nielsen, 1938). Metabolic heat production is a by-product of muscular metabolism and therefore dependent upon intensity and duration of exercise, and is far greater when an individual is hypohydrated (Pitts, Johnson and Consolazio, 1944; Ekblom *et al.*, 1970; Sawka *et al.*, 1983), not acclimatised to the heat (Shvartz *et al.*, 1979; Nielsen *et al.*, 1997) and has low aerobic fitness levels (Roberts *et al.*, 1977; Thomas, Pierzga and Kenney, 1999).

Body temperature is not a single value and is commonly divided into two compartments: (1) the external shell, which is the interface between the skin surface and the environment, and fluctuates in temperature in response to the environment; and (2) the internal core (oesophagus, rectum, gastrointestinal tract, tympanum and auditory canal), which remains relatively stable when environmental conditions initially change (Jessen, 1985; Tan and Knight, 2018). Core temperature has a greater influence on physiological thermoregulation rather than the role of skin temperature with a sensitivity for thermoregulatory responses to changes in core and skin temperature at a ratio of 9:1. For example, a 1 °C change in core temperature elicits an approximately nine-fold greater thermoregulatory response than a 1 ºC change in skin temperature (Sawka *et al.*, 2011). At the onset of exercise, core temperature initially increases rapidly due to heat production by dynamically contracting skeletal muscles and where the contribution of anaerobic energy systems predominates over

the aerobic energy system (Bangsbo *et al.*, 2001; Sawka *et al.*, 2011; González-Alonso, 2012; Nybo, Rasmussen and Sawka, 2014). During exercise, if there was no change in heat dissipation rates, when exercise exceeds the intensity equal to or greater than 80% of $\dot{V}O_{2max,}$ core temperature could increase by 1 °C every 5 min to 8 min (Armstrong and Maresh, 1998; Gleeson, 1998; Cheuvront and Haymes, 2001). This continued rise in core temperature could potentially reach life-threating levels within 10 min to 15 min of exercise (Gleeson, 1998). In an attempt to explain the thermoregulatory burden on exercise capacity and performance there are two major theories that have been proposed (see section 2.4.1.1.1). In one theory, there is evidence to suggest that exercise capacity is limited due to the attainment of a critically high core temperature of ~40 °C (Nielsen *et al.*, 1993; Cheung and McLellan, 1998a; González-Alonso, Calbet and Nielsen, 1999) while the other suggests that workload is reduced in anticipation prior to the attainment of a high core temperature in order to complete an exercise bout within the body's homeostatic limits (Marino, 2004; Lambert, St. Clair Gibson and Noakes, 2005).

2.4.1.1.1. Critical core temperature and central governor theories

There is some evidence to suggest that the attainment of a "critically" high core temperature of ~40 °C limits an individual's capacity to continue exercising, regardless of acclimation status (Nielsen *et al.*, 1993) or initial

starting core temperature (González-Alonso *et al.*, 1999). For example, Nielsen *et al.* (1993) repeatedly exposed well-trained endurance athletes to a hot, dry environmental condition (40 - 42 ºC, 10 - 15% rh) over a consecutive period of nine to twelve days. The authors reported that on completion of the heat acclimation protocol, exercise capacity time was approximately doubled from pre-acclimation baseline testing (48 ± 1.9 to 80 ± 3.3 min); however, despite this and regardless of acclimation status, participants voluntary terminated exercise with a similar (oesophageal) core temperature of ~39.8 °C. From the same laboratory, González-Alonso *et al.* (1999) investigated the influence of different initial starting body temperatures on exercise capacity during a submaximal (60% $\dot{V}O_{2max}$) cycling TTE test performed in the heat (40 °C). Before the onset of exercise, the participants were either pre-cooled (oesophageal temperature: 35.9 ± 0.2 °C), pre-warmed (38.2 ± 0.1 °C) or received no intervention (37.4 ± 0.1 °C) prior to exercise. The authors observed that exercise capacity was inversely related to starting core temperature; however, regardless of initial starting core body temperature and similar to the findings of Nielsen *et al.* (1993), participants terminated exercise at identical levels of hyperthermia (~40.1 ± 0.1 °C) (Figure 2.4). These reported findings from Nielsen *et al.* (1993) and González-Alonso *et al.* (1999) provided evidence to support the hypothesis that there is a critical core temperature at which limits the capacity to exercise in the heat.

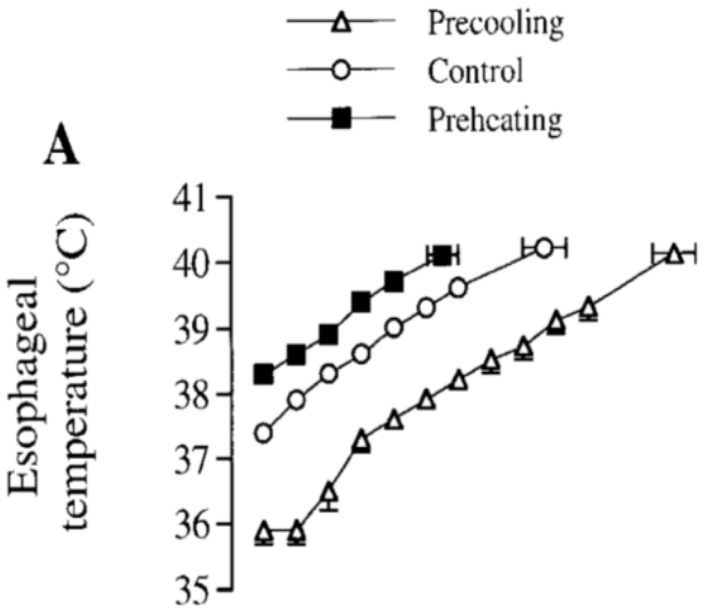

Figure 2.4. A critical core temperature consistently limits exercise in the heat (40 ºC). [Reproduced from González-Alonso *et al.* (1999), with permission].

In addition to human studies, rats were investigated to determine whether or not the critical core temperature hypothesis existed. Fuller, Carter and Mitchell (1998) measured body temperature in eight male Sprague-Dawley rats when exercising to a point of exhaustion in three different trials (23, 33 & 38 °C). The main finding was regardless of environmental temperature rats fatigued at similar abdominal temperature of 39.9 °C, even though TTE took longer in the less hot condition. These high abdominal temperatures also coincided with high hypothalamic temperatures of between 40.1ºC to 40.2 °C.

Despite these data from laboratory studies highlighting that exercise is voluntarily terminated at a core temperature of ~40 ºC in both trained and untrained individuals, there is evidence that has shown trained well-

motivated individuals to attain a core temperature above this "critical" level during sporting competitions (Pugh, Corbett and Johnson, 1967; Byrne *et al.*, 2006; Ely *et al.*, 2009; Lee *et al.*, 2010). The concept of exercise capacity and performance impairment at the attainment of a "critical" core temperature fails to recognise the complex interplay of the physiological and behavioural systems that contribute to limiting exercise capacity and performance in hot environmental conditions but rather as a continuum with from multiple physiological systems (Ely *et al.*, 2009; Meeusen and Roelands, 2010). For example, Ely *et al.* (2009) obtained rectal temperature and performance data during an 8 km track running TT and reported that out of the 17 competitive runners, 12 attained a rectal temperature of >40 ºC, with no difference in running velocity found between runners who had a rectal temperature above or below 40 ºC. Instead running velocity increased during the final 600 m when rectal temperatures would have been approaching a "critical" level. In addition to this, Byrne *et al.* (2006) had previously reported that during a half marathon race (21.1 km) 18 runners (56% of competitors) had a peak core temperature of >40 °C and 11% reached a core temperature of >41 °C, therefore, not supporting the hypothesis that a critical core temperature is the cause if premature exhaustion.

An alternative theory is known as the central governor theory, where there is evidence to support that during self-paced exercise, there is an anticipatory down-regulation in self-selected exercise intensity well before core temperature reaches this "critical" level of ~40 °C (Marino *et al.*,

2000; Tatterson *et al.*, 2000; Tucker *et al.*, 2004, 2006). Investigations into this feed-forward voluntary control theory were first explored when comparing individuals with different body compositions on running performance outcomes (Dennis and Noakes, 1999; Marino, Lambert and Noakes, 2004). Marino, Lambert and Noakes (2004) compared self-selected running velocities in 15 ºC and 35 °C environmental conditions, in six African and six Caucasian runners with a similar relative aerobic capacity (peak oxygen uptake ($\dot{V}O_{2peak}$) = 62.6 and 64.3 $mL \cdot kg^{-1}min^{-1}$, respectively) but different body masses (Africans = 59.3 kg; Caucasians = 76.6 kg). African runners completed the performance test (8 km TT) faster than the Caucasian runners when exposed to 35 °C and no difference was found in 15 °C. The authors reported similar rectal temperatures, but the relative rates of heat storage were different, which supported the theory that individuals regulate work rate to avoid heat being stored and developing exertional heat exhaustion. These findings were validated by Tucker *et al.* (2004) who observed participants to selectively down-regulate power output more rapidly in the first 6 km during a 20 km TT cycling test in hot (35 °C) than in cooler (15 °C) environmental conditions. This reduction in power output was seen before any significant increases in rectal temperature were observed and there were similar core temperature readings between hot and cooler environmental conditions during this phase of the TT. A few years later, Tucker *et al.* (2006) had eight cyclists perform three experimental trials in a cool (15 °C), temperate (25 °C) and hot (35 °C) environmental condition, where participants were instructed to cycle at a set rating of perceived exertion (16; RPE; Borg,

1982) until exhaustion. The researchers reported that self-selected power output decreased in a linear fashion with exercise duration, with the absolute rate of decline accelerated when cycling in hot environmental temperatures compared to temperate conditions, and were shown to modify the metabolic heat production. Data from these studies support the hypothesis that there is an anticipatory response that occurs, suggesting that peripheral fatigue was not the limiting factor to the reduction in exercise performance, rather it was participants "holding back" to prevent heat being stored and allowing for exercise to be completed within homeostatic limitations.

The anticipatory response is likely to be mediated by input from a number of receptors and with TT tests showing reductions in self-selected work rate well before core temperature meets a "critical" level, elevations in skin temperature may be the driving factor at mediating altered pacing strategies when exercising in the heat (Ely *et al.*, 2009, 2010; Cheuvront *et al.*, 2010; Flouris and Schlader, 2015). The skin is the largest sensory organ in the human body and therefore, changes in skin temperature and skin wetness play a critical role on the perceptual responses during exercise (Filingeri and Havenith, 2015). Cooling the skin surface by the application of a cooling stimulus (e.g. fan, menthol-gel) has shown to reduce RPE and due to a reduced perceived exertion without changes in core temperature, this has resulted in individuals being able to self-select a higher workload and subsequently improve exercise performance

(Armada-da-Silva, Woods and Jones, 2004; Mündel *et al.*, 2007; Simmons *et al.*, 2008; Schlader *et al.*, 2011a, 2011b).

The suggestion of a "critical" core temperature threshold was based upon an elevated core body temperature providing peripheral afferent feedback from thermo-sensitive receptors to the central nervous system (CNS). This feedback would result in a reduced central neural drive to the exercising muscle (Nielsen *et al.*, 2001; Nybo and Nielsen, 2001; Todd *et al.*, 2005), potentially acting as an inhibitory protective mechanism against lethal increases in body temperature (Thompson, 2006). It was demonstrated by Todd *et al.* (2005) that during maximal voluntary contractions, voluntary activation was significantly reduced when core temperature was ~38.5 °C compared to ~37 °C, where central fatigue was greater. This reduced voluntary activation and reduced work capacity, has been shown to not only coincide with the attainment of a high core temperature but also a high brain temperature, affecting the motor activity (Caputa, Feistkorn and Jessen, 1986; Nybo, Secher and Nielsen, 2002). Whilst afferent temperature feedback might reduce voluntary activation when hyperthermic, it may also be that direct elevations in brain temperature limit motor output. Due to understandable ethical and logistical reasons there are limited data on brain temperature in humans during exercise; however, it is known that the brain is sensitive to fluctuations in temperature (Wang *et al.*, 2014). Nybo, Secher and Nielsen (2002) were some of the first to calculate heat exchange within the brain during a fixed intensity exercise bout performed in a thermoneutral environment (20 °C)

and during a hyperthermic (jugular venous blood temperature; 39.5 ± 0.2 ºC) exercise condition whereby individuals wore a water impermeable suit with gloves and rain clothes. The researchers measured cerebral blood flow and temperatures of the cerebral and arterial blood by inserting catheters into the jugular vein and aortic arch (arterial), respectively. The jugular venous blood temperature was ~0.20 °C higher than aortic temperature during both conditions; however, during hyperthermia heat removal via the jugular venous blood was ~30% lower than compared to control trial. The authors concluded that this occurred due to a 20 ± 6% reduction in cerebral blood flow and impaired heat removal. This, in combination with an increase in heat production during hyperthermia, resulted in a higher brain temperature during exercise in the heat. Furthermore, participants' RPE increased in parallel with brain temperature, an important factor inducing hyperthermia-induced central fatigue during prolonged exercise and therefore, contributing to the reduced ability to exercise in the heat (Nybo, 2012).

2.4.1.2. Neurotransmitter response to exercise in the heat

The causes of heat-induced decrement in performance are expressed to be both peripheral and central factors, and therefore a complex phenomenon (Meeusen and Piacentini, 2003; Nybo and Secher, 2004). So far in this review, the mechanisms of heat-induced decrements have primarily focused on the association of heat stress on the physiological demand placed on the thermoregulatory systems. Yet to be discussed is the

influence of exercising in the heat on cerebral function, where the inability to continue exercising is likely influenced by a loss of central drive, motivation and changes in brain motor activity (Meeusen and Roelands, 2010). The decline in exercise performance was traditionally attributed to a metabolic end point, where muscle glycogen levels are depleted, plasma glucose levels are reduced, while circulating free fatty acids are elevated (Meeusen and Piacentini, 2003); however, evidence exists that declines in performance can occur from changes within the CNS (Blomstrand, Celsing, and Newsholme, 1988). Central fatigue is defined within this thesis as a progressive loss of voluntary activation or a reduced nervous excitation to the muscles and therefore, a decrease in maximal force production during exercise (St Clair Gibson, Lambert and Noakes, 2001).

The central fatigue hypothesis is based on the assumption that during prolonged exercise the synthesis and metabolism of central monoamines, are influenced (Meeusen *et al.*, 2006). In particular there is an increase cerebral serotonin (5-Hydroxytrytamine (5-HT)), dopamine (DA) and noradrenaline (NA) concentrations during exercise (Meeusen and Piacentini, 2003; Nybo, 2003; Meeusen and Roelands, 2010). The serotonergic system has been attributed to be an important modulator of mood, where increases in serotonin concentrations have been shown to contribute to feelings of lethargy and sleepiness while depressing motor neuron excitability and supressing appetite (Blomstrand, Celsing, and Newsholme, 1988). During exercise the release of adrenaline stimulates the release of free fatty acids from adipose tissue and promotes an increase

in circulating levels of tryptophan, as free fatty acids displaces tryptophan from albumin (Nybo and Secher, 2004) the longer the exercise duration the greater concentrations reported (Nybo, 2003). This unbounded tryptophan, also the precursor for the synthesis of serotonin is able to transfer across the blood-brain barrier, whereas serotonin is not. Once transferred across, the more readily available tryptophan to the serotonergic neurons increases the cerebral serotonin levels (Davis and Bailey, 1997; Nybo, 2003). In one animal study, researchers manipulated cerebral serotonin by administrating a selective serotine uptake inhibitor called fluoxetine, a serotonin agonist (8-OH-DPAT), during either a hot (35 °C) or cold (5 °C) environmental condition (Ishiwata *et al.*, 2004). Ishiwata *et al.* (2004) reported that alterations in preoptic area and anterior hypothalamus (PO/AH) serotonin levels did not mediate acute changes in the thermoregulatory system as core temperature was not reported to be different. Rather than serotonin, there is evidence to suggest that the ratio of serotonin to dopamine is the mediator of thermoregulatory adjustments and the development of reduced exercise performance (Davis and Bailey, 1997).

The neurotransmitters dopamine and noradrenaline have also been linked to impair exercise performance as they play a role in motivation and motor behaviour, with dopamine levels found to increase during prolonged exercise (Davis and Bailey, 1997; Meeusen *et al.*, 1997). Pharmacological manipulations of dopaminergic neurotransmission have been investigated more recently, where Piacentini *et al.* (2004) explored the effects of a dual

dopamine/noradrenaline reuptake inhibitor, bupropion, on exercise performance and on the hormonal response to exercise in the heat (30 °C). Despite the lack of influence of bupropion on TT performance in this study, the authors did detect a different hormonal response to occur, suggesting that the treatment did produce a central effect. In contrast to this finding, Watson *et al.* (2005) reported that cycling TT performance was improved by 9% in the heat (30 °C) when treated with bupropion than compared to non-treatment trial but had no effect on performance outcome during temperate conditions (18 °C). Seven out of the nine endurance-trained males also attained a rectal temperature of greater than 40 °C during the treatment trial compared to two out of the nine participants achieving this in the non-treatment trial (Watson *et al.*, 2005). In another study, different dosages of bupropion intake were shown to influence exercise performance, where the maximal dose (2 x 300 mg) was reported to result in well-trained male cyclists to sustain a higher power output in the heat, compared to when lower doses were ingested (2 x 150 mg & 2 x 225 mg) (Roelands *et al.*, 2012). The treatment of bupropion potentially may override the hyperthermia-induced inhibitory signals from the CNS allowing individuals to sustain and/or increase workload as perceptions of effort and thermal sensation were similar during both experimental trials. On one hand, there is evidence to support that the use of bupropion is beneficial to performance, while on the other, this could increase the risk of heat illness and therefore be detrimental to not only performance but an athlete's health.

Other pharmaceutical treatments have been considered in examining the specific role of dopamine; the use of amphetamines are known to increase extracellular dopamine, including the intake of a stimulant, Ritalin, which has received some attention in an attempt to elevate dopamine concentrations (Fukui *et al.*, 2003; Roelands *et al.*, 2008). The drug Ritalin is used to treat a neurobehavioral disorder called Attention Deficit Hyperactivity Disorder. The use of a stimulant such as methylphenidate (i.e. Ritalin) inhibits the reuptake of dopamine and norepinephrine, and therefore, increases the concentration of these neurotransmitters in the brain. The administration of Ritalin during the same exercise protocol used in the bupropion studies, was shown to enable participants to maintain a higher power output and finish the TT 16% faster in hot (30 °C) conditions compared to the non-treatment trial (Roelands *et al.*, 2008). Increasing dopamine concentrations after Ritalin intake did not improve TT performance in temperate conditions (18 °C), suggesting that dopaminergic neurotransmission plays a role in central fatigue only when performed in hot environmental conditions (Roelands *et al.*, 2008). In a follow up study, the use of Reboxetine, which is a specific noradrenaline reuptake inhibitor, was investigated. In contrast to the use of Ritalin, Reboxetine decreased performance by 10% in temperate conditions (18 °C) and by 20% in hot conditions (30 °C) (Roelands *et al.*, 2008). The enhancements of noradrenaline concentrations were detrimental to performance, even though this neurotransmitter is thought to play a role in the control of arousal, consciousness and reward. The manipulation of neurotransmitters from these studies, suggests that central

neurotransmitter homeostasis has a key role to play in the onset of fatigue during exercise in the heat.

2.4.1.3. Cardiovascular challenges in response to exercise in the heat

The cardiovascular system can be severely challenged when exercise is combined with heat stress (Crandall and González-Alonso, 2010). As early as 1916, Lee and Scott indicated that a possible mechanism for premature fatigue during exercise in heat was due to the circulatory adjustments that "drafted blood away from the brain and the muscles to the skin" (cited in Nybo, Rasmussen and Sawka, 2014; Périard *et al.*, 2016). The extent of the challenge imposed upon the cardiovascular system is highly influenced by the intensity and duration of exercise, training, acclimation and hydration status of the individual (Sawka *et al.*, 1983; Crandall and González-Alonso, 2010), where the greatest cardiovascular challenge has been reported in untrained (Rowell *et al.*, 1966), unacclimated and hypohydrated individuals (Sawka *et al.*, 1983).

One of the initial responses when exposed to ambient air temperatures equal to or greater than skin temperature, is an instant increase in skin temperature. The skin represents the interface between the body and the environment and serves as a sensory organ that provides thermal information and feedback signals to the thermoregulatory system (Romanovsky *et al.*, 2009; Romanovsky, 2014). The initiation of exercise causes an acute reflex vasoconstriction in the skin, a response that occurs

to redirect blood flow from non-active vascular beds to the active skeletal muscles, which may in turn increase core temperature (Kenney and Johnson, 1992). The extent of this redistribution and vasomotor tone is dependent on the intensity of exercise, whereby higher intensity exercise delays the onset of vasodilation (Taylor *et al.*, 1988). The rise in core temperature initiates a thermoregulatory efferent response received from afferent signals in an attempt to maintain core temperature via active cutaneous vasodilation (Mekjavic and Eiken, 2006). Through thermoregulatory vasomotor reflexes and heat stress responses, large increases in skin blood flow occur because the venous bed of the skin is large and dilates, increasing convective heat transfer from the active skeletal muscle and deep tissues to the body surface (Charkoudian, 2003; Sawka *et al.*, 2011). Skin blood flow has been recorded to increase several-fold from normal resting values of ~300 $mL \cdot min^{-1}$ to ~7500 $mL \cdot min^{-1}$ (Rowell *et al.,* 1969; Rowell, 1986). Therefore, one of the primary challenges for the cardiovascular system is to provide sufficient cardiac output to the active skeletal muscle to meet the energetic demands for muscular activity (oxygen) and to the skin to meet the demands of temperature regulation (González-alonso, Crandall and Johnson, 2008; Périard *et al.*, 2016). Furthermore, CNS function must also be regulated, by maintaining mean arterial blood pressure to ensure that there is adequate perfusion pressure to all organs (Joyner and Casey, 2015).

In an attempt to meet the demands of cardiac output during strenuous exercise performed in a hot environment, HR increases because stroke

volume and mean arterial pressure decline (Rowell *et al.*, 1966; Périard *et al.*, 2011). In a classic study, Rowell *et al.* (1966) compared two environmental temperatures (46 ºC vs 26 ºC) on cardiovascular responses during moderate exercise and reported stroke volume (-20 mL) and cardiac output (1.0 – 1.2 $L \cdot min^{-1}$) to be lower when exercising in hot (43 ºC) compared to temperate (23 ºC) conditions. In another study, González-Alonso *et al.* (1997) investigated the effects of hyperthermia on cardiovascular function in the heat (35 ºC) without or with ~4% hypohydration. Hyperthermia (oesophageal temperature ~39.3 ºC) alone during 30 min exercise bout performed at ~72% $\dot{V}O_{2max}$, was reported to maintain cardiac output by compensatory increase in HR by 5 ± 1% despite a decrease in stroke volume by 8 ± 2%. Whilst it is often considered that the thermoregulatory demand of a high skin blood flow is the cause of a compromised stroke volume and cardiac output, more recent data have challenged this viewpoint by demonstrating that, before exhaustion during maximal exercise in trained individuals, stroke volume and cardiac output are higher in the heat compared to control conditions (González-Alonso and Calbet, 2003). This has been supported more recently by Chou *et al.* (2019). When the cardiovascular system cannot meet the demands of the muscle and the skin, the maintenance of blood pressure takes precedence over skin blood flow, which impairs the ability to lose heat and therefore, increases the rate of hyperthermia (Rowell, 1986; Nadel, 1979; Casa, 1999).

2.4.1.4. Gastrointestinal responses during heat stress

A number of surveys have shown athletes have a high a prevalence of gastrointestinal (GI) complaints, with 30% to 90% of these athletes experiencing GI problems related to training and racing (Pfeiffer *et al.*, 2012; De Oliveira, Burini and Jeukendrup, 2014). In the 1970s, Bill Rodgers, a former American recorder holder of the marathon expressed that, "More marathons are won or lost in the porta-toilets than at the dinner table," (S79, De Oliveira, Burini and Jeukendrup, 2014). GI symptoms, such as nausea, vomiting, abdominal angina and bloody diarrhoea (Peters *et al.,* 1999; Ter Steege *et al.,* 2008) have been considered to be multifactorial with the prevalence of these symptoms depending upon the exercise intensity, exercise type, environmental conditions, sex and age. Lower GI incidences have been reported to occur more commonly during running exercises, where younger individuals (<20 years old) and women have a higher incidence rate, than compared to older individuals and males (Keeffe *et al.*, 1984; Peters *et al.*, 1999). Peters *et al.* (1999) surveyed runners (n = 199), cyclists (n = 197) and triathletes (n = 210) and reported runners to experience a higher prevalence of lower GI symptoms (71%) compared to cyclists who experienced both upper (67%) and lower (64%) GI symptoms. It was therefore unsurprising that Peters *et al.* (1999) reported that triathletes had prominently upper GI symptoms during the cycling stage (52%) and lower GI symptoms (79%) during the running stage. More recently, Stuempfle and Hoffman (2015) interviewed ultra-marathon runners during a 161 km race at four location points (48 km, 90 km, 126 km and at the finish) and were asked

to rate the severity and frequency of each GI symptom. The most commonly reported symptom was nausea (60%) and at least 16 out of the 20 finishers reported to experience a GI symptom during the race at some point. Even though 60% of the runners experienced nausea, this GI symptom did not impact upon finishing time (with nausea 26.9 ± 2.7 h; without nausea 26.8 ± 2.1 h; $P > 0.99$).

The higher prevalence of GI symptoms during exercise under heat stress has limited evidence to show that this response occurs in line with an increase in circulating endotoxin called lipopolysaccharide (LPS). LPS is a major component of the outer membrane of a gram-negative bacteria that harbours within the gut, which consists of molecules that can be divided into three parts; lipid A, core polysaccharide and O-antigen repeats. The hydrophobic lipid section, lipid A is responsible for the major bioavailability of the endotoxin. Small amounts of LPS that transfer across from the gut into the systemic circulation are cleared rapidly, however, when the rate of clearance cannot keep up with the rate of translocation from the GI tract into the lumen, endotoxemia develops. This response can occur when the epithelial cell walls have been compromised and an increase in tight junction permeability allows for a greater influx of LPS into the circulation. In cell culture studies, heat stress leading to hyperthermia, has been shown to increase tight junction permeability (Dokladny, Moseley and Ma, 2006; Dokladny *et al.*, 2008). Even though there is some evidence showing hyperthermia induces intestinal permeability in cell culture studies, there is limited evidence available when examining human studies. In one study,

Marchbank *et al.* (2011) reported intestinal permeability to increase in a double-blinded placebo-controlled trial after twelve participants performed 20 min of running on a treadmill with 1% incline at 80% of $\dot{V}O_{2max}$, rectal temperature was reported to rise moderately to 38.4 ºC. In more recent human studies that explored intestinal permeability, it was reported that core temperatures between 38.2 ºC and 39.4 ºC increased intestinal permeability (Lambert, 2008; Van Wijck *et al.*, 2011; Shing *et al.*, 2014).

Permeability has previously been expressed to relate to the property of a membrane that allows unmediated diffusion to occur (Bjarnason, Macpherson and Hollander, 1995). Therefore, the increased intestinal permeability allows for the translocation of LPS to enter the systemic circulation from the lumen (Van Wijck *et al.*, 2012). This release of LPS into the circulation stimulates an innate immune response, which triggers a cascade of inflammatory responses. When LPS is detected it activates monocytes/macrophages, which results in a secretion of pro-inflammatory cytokines including tumour necrosis factor a (TNF-α), interleukins (IL)-1 and IL-6. The liver is the first organ that encounters the translocation of LPS molecules from the intestinal lumen to the blood stream via the portal circulation and is continuously exposed to a variety of antigens exiting the gut into the portal circulation. On this note, the liver plays a crucial role in the immune response and clearance of bacteria and toxic molecules. Kupffer cells are hepatic macrophages that have key roles in the clearance of endotoxins from the circulation. In response to LPS, Kupffer cells are

directly activated, secreting cytokines including IL-1 and IL-6 and the pro-inflammatory cytokine TNF-α. This is an important host defence mechanism and therefore, plays a fundamental role in the detoxification of LPS.

An increase in circulating levels of LPS has been observed post marathon, ultra-marathon and triathlon races (Bosenberg *et al.*, 1988; Brock-Utne *et al.*, 1988; Camus *et al.*, 1998; Jeukendrup *et al.*, 2000) and has been shown to be correlated with GI symptoms (Jeukendrup *et al.*, 2000) and heat stroke (Leon and Helwig, 2010). In one study, Pals *et al.* (1997) examined the effect of running intensity (40%, 60% and 80% of $\dot{V}O_{2max}$) on intestinal permeability in six physically active volunteers in a moderate temperature environment (22 ºC, 50% rh). The permeability of the gut was reported to be influenced by the intensity of exercise, as intestinal permeability increased after 60 min of high intensity exercise (80% $\dot{V}O_{2max}$). LPS concentrations were not measured, and therefore the authors were unable to conclude whether the increase in intestinal permeability during exercise resulted in higher circulating concentration of LPS. To date, the limited evidence available makes it challenging to clearly identify whether heat stress or exercise intensity per se or the combination of both play a key role in increasing intestinal permeability. The exact mechanism/s that underpin the increased intestinal permeability remains unclear and is likely influenced by an interplay of the physiological consequences of exercise, which includes an increase in core temperature, hypoxia, free radicals, and exercise intensity. At rest, the splanchnic organs receive ~20% of the cardiac output and

consume only 10% to 20% of the available oxygen (Rowell *et al.*, 1964; Ter Steege and Kolkman, 2012). However, during exercise several reflex adjustments take place to compensate for the demand of blood flow to the muscle and the skin. One of the adjustments to occur is a reduction in blood flow to the splanchnic and renal region to allow for cardiac output to be redistributed. The magnitude of reduction to these regions during exercise is in proportion to exercise intensity and heat stress (Rowell *et al.*, 1966). In order to maintain arterial blood pressure, the sympathetic nervous system (SNS) causes an increase in resistance to the splanchnic vasculature resulting in the diversion of blood away from the splanchnic region leading to a reduced perfusion of internal organs (Rowell *et al.*, 1966; Rowell, 1973; Kvietys and Neil Granger, 2014). Reductions in splanchnic perfusion have been reported to occur within 10 min of strenuous exercise and can decrease by up to 80% during maximal exercise (Rowell *et al.*, 1964; Rowell, 1974; Otte *et al.*, 2001; Rehrer *et al.*, 2001).

2.4.1.5. Summary of the proposed physiological mechanisms for fatigue in the heat

In summary, exercising in the heat is associated with an increase in thermoregulatory strain which challenges the cardiovascular, neuromuscular, and the central nervous system. During exercise capacity tests, the voluntary termination of exercise has typically coincided with the attainment of a core temperature of ~40 ºC in both humans and animal studies (Nielsen *et al.*, 1997; Fuller, Carter and Mitchell, 1998; González-

Alonso *et al.*, 1999). However, in performance studies, self-selected exercise intensity is impaired due to a down-regulation in self-selected workload prior to the attainment of a high core temperature, a response to occur in anticipation, acting as a protective mechanism to reduce heat storage and allow for exercise to be completed within homeostatic limits (St Clair Gibson, Lambert and Noakes, 2001; Tucker *et al.*, 2004; Lambert, St. Clair Gibson and Noakes, 2005). The increased thermoregulatory burden during exercise under heat stress places an additional strain on the circulatory system to not only meet the energetic demands of blood flow to the muscle but also to the skin to meet the thermoregulatory demands. One of the cardiovascular adjustments to occur is the redistribution of blood away from the splanchnic region and an increase in HR to meet the demands of cardiac output. The conflict between these two systems results in a number of physiological implications, with one being the integrity of the intestinal epithelial cell walls (Pals *et al.,* 1997) resulting in the translocation of toxic samples, such as LPS, from the intestinal lumen into the internal environment (Lambert, 2008). Together these regulatory systems are in competition, resulting in an increased risk of heat being stored, exacerbated by the duration and intensity of exercise, subsequently impairing exercise performance.

2.4.2. Behavioural responses during exercise in the heat

Until this point in the literature review, the focus has been on the autonomic physiological systems and the challenges that are imposed upon

the athlete when exercising in the heat; however, full accountability cannot be placed upon the physiological systems to be the limiting factor to exercise performance. It is likely a complex interaction between the physiological and behavioural systems that govern exercise performance in the heat. Behavioural thermoregulation has been proposed to be the first line of defence in an attempt to maintain thermal equilibrium during rest and exercise (Flouris and Schlader, 2015). Weiss and Laties (1961) were one of the early researchers to highlight that behavioural thermoregulation is one of the fundamental mechanisms by which organisms regulate thermal homeostasis. An example of one of these thermal behaviours may include a change in power output or running velocity, whereby a reduction would reduce the metabolic cost of the exercise task and therefore, reduce metabolic heat production, resulting in less heat being stored (Schlader *et al.*, 2011a; Schlader, Stannard and Mündel, 2011; Flouris and Schlader, 2015). Other examples of thermal behaviours that have been reported to occur are the modification of fluid consumption and clothing alterations, whereby the exercising individual may take a layer of clothing off. In the upcoming sections, the main focus will be the influence of environmental temperatures on RPE (Borg, 1982) thermal comfort (TC) (Gagge, Stolwijk and Hardy, 1969) and sensation (TS) (Young *et al.*, 1987) (*Appendix D1-3*).

2.4.2.1. Perceived exertion during exercise in the heat (Appendix D1)

There is an abundance of evidence that has shown that RPE is rated higher when exercising at the same relative exercise intensity, in a hot compared to temperate environmental condition (Galloway and Maughan, 1997; Tucker *et al.*, 2006; Zora *et al.*, 2017). The Borg scale (Borg, 1982), is the most commonly used tool to measure subjective feelings of effort and exertion during exercise (Zora *et al.*, 2017). Perceived exertion is one of the key drivers that modulates exercise intensity and pacing strategies adopted during self-paced exercise (Tucker *et al.*, 2006; Borg *et al.*, 2018). In one study, Maw, Boutcher and Taylor (1993) reported that when participants cycled at the same relative intensity in a hot environmental condition (40 °C) they rated a significantly higher RPE, felt worse and reported a greater TS than when exercising in a cool (8 ºC) and temperate environmental condition (24 °C). In another study that systemically investigated 10 °C differences in ambient air temperature, RPE was rated the highest when exercising during a hot (31 °C) environmental condition compared to when exercising in moderate (21 °C) and cool environmental conditions (11 °C) (Galloway and Maughan, 1997).

During a performance test that clamped RPE to the value of 16, Tucker *et al.* (2006) observed a greater rate of decline in self-selected power output when cycling in a hot (30 °C) compared to temperate (25 °C) and cool (15 °C) environmental conditions. During the first few minutes of exercise

self-selected power output declined rapidly and this response was interpreted that the regulation of exercise intensity was in response to afferent feedback relating to the rate of heat storage and to prevent excesses heat being stored, which has also been observed by others (Tatterson *et al.*, 2000; Marino, 2004; Tucker *et al.*, 2004). A higher RPE was observed to occur in the aforementioned studies in the absence of increased physiological strain or differences in physiological states between trials (Tucker *et al.*, 2006; Crewe, Tucker and Noakes, 2008). Providing evidence to support that the rate of heat storage is mediated by anticipatory reduction in exercise intensity which is mediated through RPE and other perceptions of thermal discomfort in an attempt to prevent core temperature exceeding homeostatic limits and therefore acting as a protective mechanism (Tucker, 2009).

The conscious and unconscious perception of thermal stress during exercise has been investigated, where the influence of deception of a certain variable such as the end point of exercise (Eston *et al.*, 2012), environmental temperature and perception of core temperature have been explored (Castle *et al.*, 2012; Borg *et al.*, 2018). During one study, awareness of environmental and core temperature was shown to influence 30 min cycling TT performance (Castle *et al.*, 2012). When participants were deceived and told that the environmental conditions they were exercising in (26 ºC) and their core temperatures (-0.3 ºC) were actually lower than the true environmental conditions (31 ºC) and core temperature readings, mean power output was greater than compared to when not

deceived. Castle *et al.* (2012) reported a lower RPE when deceived, allowing for a higher selection of power output during TT. With RPE regarded as a key psychophysiology cue for regulating exercise intensity, not all performance improvements may occur in the presence of a lower reported RPE (Tucker, 2009). More recently, Borg *et al.* (2018) explored whether perception of environmental temperature influenced RPE response when participants were either provided with accurate feedback or were deceived of environmental temperatures. RPE was similar in all hot (33 °C) trials that were performed at a fixed power output, regardless of the information provided. This is one of the first studies to provide evidence that RPE in trained cyclists was not mediated by awareness of environmental temperature. While the influence of RPE on the regulation of exercise intensity can account for some of the psychological inputs in moderately trained individuals, it appears there are other conscious inputs that are integrated in this complex regulatory system in highly trained individuals. These include the interplay of TS and TC in mediating the anticipatory adjustments in exercise intensity and acting as a protective mechanism in preventing the athlete from exercising beyond homeostatic limits, which are discussed below.

2.4.2.2. Thermal perceptions during exercise in the heat (Appendix D2 - 3)

Thermal comfort (TC) has been defined as an individual's subjective indifference to the environment, whereas TS relates to the relative

intensity of the temperature that is being sensed (Schlader *et al.*, 2009; Flouris and Schlader, 2015). Perceptual responses are related a sensation of a warm/hot thermal stimulus in response to ambient, humidity, skin and core temperatures (Winslow, Herrington and Gagge, 1937; Gagge, Stolwijk and Hardy, 1969). The influence of these thermal perceptions on exercise performance remains a complex issue with little consensus (Cheung, 2010). There is disparity that exists, which can vary greatly within individuals depending on physiological factors (i.e. fitness ability, body fat composition). Thermal disturbances are rapidly detected by thermoreceptors in the skin, which relay information and act as a controller of thermoregulatory behaviour (i.e. power output increase/decrease) (Schlader, Stannard and Mündel, 2010). In one study, Schlader *et al.* (2009) identified it was the temperature of the skin that initiated the thermoregulatory behaviour to move between either a hot (45 °C) or cool (10 °C) environment and this behaviour effectively maintained core temperature as no differences were reported between trials. Changes in skin temperature were also shown to influence power output during exercise, the rate of decline in power output in hot conditions was observed to occur in the presence of a hot skin temperature (Tucker *et al.*, 2006; Duffield *et al.*, 2010; Ross *et al.*, 2011; Schlader *et al.*, 2011a). Therefore, it seems that skin temperature plays an important role in the feedback control system, which mediates the selection and maintenance of power output or running velocity during exercise in anticipation to prevent heat being stored (Levels *et al.*, 2012).

2.4.2.3. Summary of behavioural responses during exercise in the heat

In summary, prior to any changes in core temperature voluntary reductions in self-selected workload occur during exercise in the heat (Tatterson *et al.*, 2000; Tucker *et al.*, 2004). This is likely mediated by thermal perceptions (TC & TS), which in turn modulates perception of effort (RPE). It is well-accepted that RPE increases linearly as exercise progresses and independent of environmental temperature exercise is terminated at near maximal levels of RPE (Galloway and Maughan, 1997; González-Alonso *et al.*, 1999). However, during exercise performed under heat stress the rate of rise in RPE is accelerated (Crewe, Tucker and Noakes, 2008), which is in response to an elevated skin temperature, therefore, increasing thermal discomfort. Interventions to reduce and/or offset perceived thermal strain before and during exercise in the heat are of high importance in allowing athletes to maintain exercise intensity and minimise the impairments in exercise performance that are associated with heat stress.

2.5. Strategies to delay the onset of hyperthermia-induced fatigue during exercise in the heat

A number of interventions have been investigated in order to reduce the physiological and perceptual strain associated with a rise in core temperature. The three main interventions that have been investigated are hydration, cooling and HA protocols. During the 2015 International

Association of Athletes Federation (IAAF) World Championships in Beijing (China), 957 athletes completed a pre-competition questionnaire asking them to identify the heat stress prevention and heat alleviation interventions (hydration, pre-cooling and acclimation) that they planned to use (Périard *et al.*, 2017). Of the athletes questioned, 96% had a pre-planned fluid consumption intervention, 15.3% specifically trained in the heat (with the length of exposure varying between 17 ± 10 days) and approximately half of the athletes (52.4%) had one pre-arranged cooling intervention. These three key interventions will be discussed in the upcoming subsections.

2.6. Hydration

2.6.1. Fluid regulation and body water

Water is the most abundant molecule and major constituent within the human body, making up between 45% to 70% of total body mass, and is tightly regulated in order to maintain homeostasis (Institute of Medicine (IOM), 2005; Jéquier and Constant, 2010). The varying differences in percentages reported is because of differences in body fat composition, as fat-free mass is ~70% to 80% water, while adipose tissue is 10% water (Sawka *et al.,* 2005). Insufficient or excess water intake changes cellular volume and effects cellular functions (Armstrong *et al.*, 2010). Water is essential for cellular homeostasis and depending on the osmotic gradient will transport nutrients to cells and remove waste away from the cells. Water therefore acts as the medium in which all transport systems function

allowing the exchange between cells, fluid spaces and capillaries (Häussinger, 1996; Jéquier and Constant, 2010) a process known as osmosis. As well as a carrier, water acts as a physiological lubricant (e.g. salvia, digestive & respiratory tract) and shock absorber for joints during walking or running, which is important for protection of the brain and the spinal cord (Jéquier and Constant, 2010).

Water is distributed into two main fluid spaces known as the intracellular fluid (ICF) and the extracellular fluid (ECF) compartments, which contain ~65% and ~35% of total body water, respectively (Sawka *et al.,* 2005). The extracellular fluid can be divided into two primary constituents; the interstitial fluid and blood plasma spaces, which are separated by highly permeable capillary membranes. The difference in physical properties between the two cellular compartments are the presence of different major cations and anions. In the ECF, the cation sodium (Na^+) and anions chloride and bicarbonate are in abundance, while in the ICF, the presence of the cation potassium (K^+) and inorganic phosphates predominate (Asim *et al.*, 2019). Sawka, Cheuvront and Kenefick (2015) gave an example, that for an average 70 kg male with a total body water content of 60% body mass (42 L), the intracellular compartment would have ~28 L and the extracellular compartment would have ~14 L of water, which would be divided into 10.5 L in the intestinal fluid and 3.5 L in the intravascular fluid. Throughout this thesis euhydration is defined as normal state of body water content which oscillates during the day due to water losses and gains, whereas, dehydration is the process of water being lost and hypohydration

is referred to as a body-water deficit (Sawka, 1992). Water loss occurs on a daily basis through urine, sweat, respiration, faeces and the skin and gained from food, fluid ingestion and metabolic water formation processes.

2.6.2. Water loss via sweating during exercise in the heat

During exercise where ambient air temperature exceeds the ability to dissipate heat via dry heat exchange mechanisms, evaporative heat loss and therefore, sweating becomes the primary means to dissipate heat (Sawka, 1992). Sweat losses vary from individual to individual (1 $L \cdot h^{-1}$ to 4 $L \cdot h^{-1}$) and depends upon a number of variables including; fitness level, environmental conditions (i.e. temperature, humidity, air velocity), and metabolism (Sawka, Cheuvront and Kenefick, 2015). The higher the thermal load from the environment, the greater the reliance on evaporative heat loss from sweating, leading to a greater amount of total body water lost in addition to and some salt (Merry, Ainslie and Cotter, 2010). Typically, fluid intake during exercise is insufficient to match fluid losses, resulting in hypohydration occurring during prolonged exercise.

Sweat secreted during exercise decreases plasma volume (i.e. hypovolemia) and as sweat is typically hypotonic relative to plasma (i.e. hyperosmotic) this will result in an increase in osmotic pressure in the ECF. In order to maintain osmotic equilibrium between the ICF and ECF, this increase in pressure results in a movement of fluid from the larger intracellular space (Cheuvront and Kenefick, 2014). The outcome of this

is known as hypertonic hypovolemia, which has a number of physiological and behavioural responses that mediate the impaired exercise capacity and performance outcome associated with hypohydration, as highlighted in section 2.2.1.

The figure below (Figure 2.5) provides an illustration of the approximation of hourly sweating rates during running based upon data collected during laboratory investigations. As air temperature, humidity and running velocity increase, higher sweat rates are reported. It is not uncommon to report athletes to finish a race hypohydrated, as it is challenging to consume the volume of fluid required to balance the volume of sweat output during exercise in the heat (Sawka, 1992). This has been referred to as both voluntary and involuntary dehydration (Greenleaf and Sargent, 1965; Greenleaf, 1992).

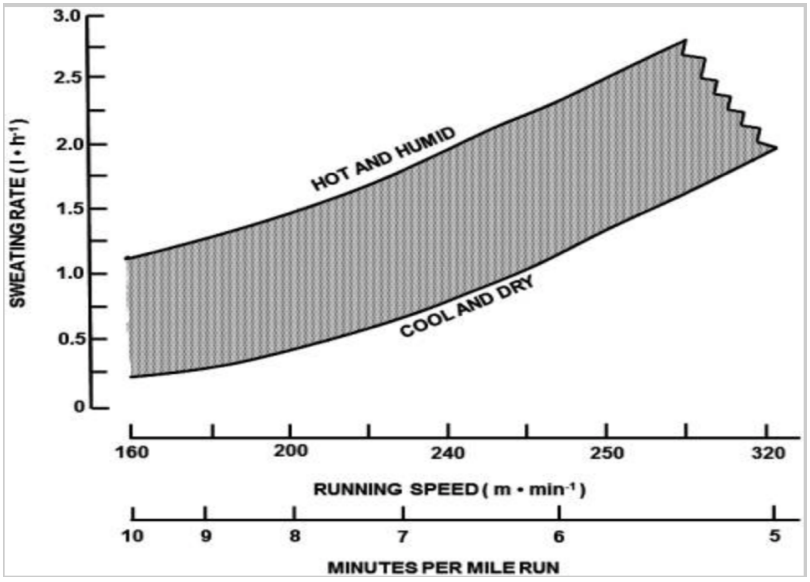

Figure 2.5. The approximation of hourly sweating rates ($L \cdot h^{-1}$) for runners at different running paces ($m \cdot min^{-1}$ or min per mile) and environmental conditions (hot and humid, cool and dry). [Reproduced from Sawka (1992), with permission].

2.6.3. The effect of hypohydration on exercise performance

A common belief during the early 1900s, was that fluid consumption during a marathon race was a sign of weakness and lack of fitness ability, which enforced athletes to abstain from consuming fluid before and during running events (Murray and Udermann, 2003). Thereafter, during the 1940s, evidence on the adverse effects of hypohydration on exercise capacity in the heat started to emerge (Pitts, Johnson and Consolazio, 1944). From then on, the detrimental consequences of hypohydration of ~2% to 3% loss of body mass on exercise capacity and performance have been extensively reported from laboratory-controlled studies in the heat (Sawka *et al.*, 1984; James *et al.*, 2017; Funnell *et al.*, 2019) compared to when exercising in temperate environmental conditions or in a euhydrated state (Cheuvront *et al.*, 2005). For example, Sawka *et al.* (1985) compared the effects of euhydration to three graded hypohydrated experimental trials (3%, 5% and 7%) during a walking exercise bout (140 min) at a low fixed intensity speed in a hot dry environmental condition (49 °C, 20% rh). The eight participants were able to complete both the euhydrated and 3% hypohydrated trial, but only seven and two out of the eight participants were able to complete the 5% and 7% hypohydrated trials, respectively. The authors concluded that hypohydration caused an earlier onset of exercise-induced tiredness and therefore, limited an individual's capacity to continue exercising in the heat. This evidence was later supported by

Sawka *et al.* (1992), who found that exercise duration was reduced from 121 min in the euhydrated trial to 55 min, when participants became gradually hypohydrated (body mass loss = ~8%) during walk to exhaustion exercise in the heat (49 °C).

Cheuvront *et al.* (2005) investigated the effect of 3% hypohydration upon a 30 min performance (TT) test after participants had completed a 30 min preload exercise at 50% $\dot{V}O_{2max.}$ The authors reported that hypohydration impaired performance by 8% when exercising in temperate (20 °C) compared to when exercising in cold (2 °C) environmental conditions. Kenefick *et al.* (2010) investigated the effect of 4% hypohydration on aerobic performance in four ambient conditions (10 ºC, 20 ºC, 30 ºC & 40 °C). Hypohydration was found to impair aerobic performance at a greater percentage with increasing environmental temperatures. When comparing the euhydration trial to hypohydration, 15 min TT performance was impaired by 3% (10 °C), 5% (20 °C), 12% (30 °C) and 23% (40 °C). The impairments in performance seen at 20 °C were similar between Kenefick *et al.* (2010) (~5%) and early findings by Cheuvront *et al.* (2005) (~8%).

The conclusion that hypohydration impairs exercise capacity and performance is strongly based upon laboratory-controlled studies; however, there is emerging evidence that this may not apply under real-world racing conditions (Goulet, 2012). When comparing field data to laboratory findings, it is often reported that the faster runners finish with the greatest level of hypohydration (Pugh, Corbett and Johnson, 1967;

Wyndham and Strydom, 1969; Sharwood *et al.*, 2004; Zouhal *et al.*, 2011). In marathon runners, Pugh, Corbett and Johnson (1997) reported that the winner (2 h 28 min) had lost 6.9% of his body weight, with the top four athletes finishing the race all having lost ~5.8% of their body weight. It was also observed that during an Ironman triathlon there was a significant relationship between finishing times and body mass losses (Sharwood *et al.*, 2004). Sharwood *et al.* (2004) reported that the greatest body mass losses were also from athletes that were the fastest. In more recent findings, evidence from Zouhal *et al.* (2011) supported both Pugh, Corbett and Johnson (1997) and Sharwood *et al.* (2004) by reporting a significant linear relationship between the percentage of body mass lost and race finish time. These studies are just some of the supporting evidence that highlights body mass loss of greater than 2% does not impair exercise performance within racing real-world conditions.

Despite substantial evidence from laboratory-controlled studies highlighting that hypohydration greater than 2% has consequences on exercise capacity and performance, data from some laboratory-controlled and field studies do not always support this consensus (Cheung *et al.*, 2015; Wall *et al.*, 2015) and has been debated (Goulet, 2011; Cotter *et al.*, 2014). While there is clear mechanistic basis for how hypohydration might impair exercise capacity and performance in the heat (reviewed in the upcoming subsections 2.6.5 & 2.6.6), methodological techniques used to induce hypohydration within some of these studies has raised a number of concerns and potentially may have confounded these performance

outcomes reported. In the upcoming section, limitations of methodologies used will be discussed.

2.6.4. The methods used to induce hypohydration

The methods used to induce hypohydration reported within the literature, may not reflect or replicate the typical hypohydration consequences on the physiological and perceptual systems. Firstly, from a physiological standpoint, different methodologies used to induce hypohydration have altered the redistribution of water between the ICF and the ECF compartments. For example, diuretic-induced hypohydration, results in iso-osmotic hypervolemia where a greater amount of fluid is lost from the extracellular compartments, which is indifferent to water deficits from sweating.

Secondly, the overtness of the methods used to induce hypohydration (e.g. fluid restriction) has meant that participants are likely aware and not blinded to the experimental protocol (e.g. euhydrated or hypohydrated). This awareness potentially contributes to a nocebo effect on performance outcomes when investigating hypohydration, as participants may have an expectancy of hypohydration (McClung and Collins, 2007). As well as an awareness of the true experimental aim, the restriction of fluid often results in an increased sensation of thirst, due to an increased secretion of vasopressin in response to hyperosmotic hypovolemia (Sawka *et al.*, 1985). The sensation of thirst has been shown to be one of the driving factors modulating exercise intensity, acting as part of the anticipatory

regulatory system (Sawka and Noakes, 2007; Noakes, 2010). However, with the development of new methodology, where thirst is controlled but hypohydration still remains, exercise performance is reported to decrease in the absence of thirst (Adams *et al.*, 2018). New methodological approaches have been investigated, with both Wall *et al.* (2015) and Cheung *et al.* (2015) in the same year investigating the hypohydration status on exercise performance, through the manipulation of hydration status via intravenous saline solutions. While the method, successfully blinded participants to their true hydration status, the typical physiological consequences of hypohydration were dissimilar to what would occur from exercise-induced sweating. Replacing sweat losses with the infusion of isotonic saline solution resulted in plasma volume remaining elevated (i.e. hyperosmotic), compared to hypertonic hypovolemia which occurs from exercise-induced hypohydration.

Recently, both James *et al.* (2017) and Funnell *et al.* (2019) used a nasogastric tube as a methodological technique to blind participants of their true hydration status while inducing hypohydration (2% to 3%). In both these studies an exit interview was conducted, and it was revealed that participants were unable to identify the true aims of the study. Therefore, the use of a nasogastric tube has been shown to be a successful methodological approach to use in blinding participants of their true hydration status.

2.6.5. Physiological responses to hypohydration

2.6.5.1. Thermoregulatory responses to hypohydration

One of the physiological consequences with exercise-induced hypohydration is the inability to dissipate heat due to an impaired sweating ability, which results in a rise in core temperature exacerbating the risk of hyperthermia (Sawka *et al.*, 1984; Sawka, 1992), compared to when fluid is replaced during exercise (Pitts, Johnson and Consolazio, 1944; Ekblom *et al.*, 1970; Sawka *et al.*, 1985). As the magnitude of hypohydration increases, a concomitant graded elevation in core temperature during exercise is observed (Sawka, 1992). During one investigation, Sawka *et al.* (1985) reported that there was a reduced sweat rate for a given core temperature with increasing severity of hypohydration. These findings, and others, have shown that increasing hypohydration results in an inability to sweat and therefore, impairing the ability to dissipate heat, resulting in heat gained – as noticed by the rise in core temperature. In more recent findings, Buono and Wall (2000) confirmed the earlier findings of Sawka *et al.* (1985) and reported a similar rise in core temperature (~0.16 °C) for every 1% decrease in body mass during exercise in the heat (33 °C). Buono and Wall (2000) also compared the consequences of exercising while hypohydrated during temperate conditions (23 °C) and observed that for every 1% decrease in body mass, core temperature (rectal) increased by 0.08 °C. This finding supports earlier observations where Greenleaf and Castle (1971) reported a similar elevation in core temperature (rectal) (0.10 °C per % decrease) during

exercise in a temperate condition (24 °C). Data from these studies highlight that hypohydration increases core temperature during exercise performed in temperate conditions but the severity of increase in core temperature is greater during exercise in the heat, where evaporation of sweat is the primary heat exchange mechanism to dissipate heat.

Large variations in core body temperature responses to hypohydration have been reported due to differences in participant populations, environmental conditions, exercise type/intensity and methodologies used to induce hypohydration and the measurement site for core body temperature. When a higher intensity of exercise (74% $\dot{V}O_{2max}$) has been used, elevations of 0.40 °C have been observed for every 1% decrease in body mass (Gisolfi and Copping, 1974). In one study, Montain *et al.* (1998) determined the impact of exercise intensities (25%, 45% and 65% of $\dot{V}O_{2max}$) on the thermoregulatory and cardiovascular strain at three different hydration statuses (0%, -3% and -5% body mass losses) during heat stress. It was observed that elevations in core temperature (0.12 °C per % decrease) were a result of the magnitude of percentage of body mass loss, with exercise intensity having little effect on thermoregulatory strain.

At first it was questioned whether the hypohydrated-mediated heat storage was a result of either an increase in metabolic heat production or the inability to dissipate heat (Sawka, 1992). Evidence points towards the rise in core temperature observed when hypohydrated, is due to plasma hyper-osmolality and plasma hypovolemia. Hypohydration can impair

evaporative heat loss through a delayed sweating onset (Strydom and Holdsworth, 1968), sensitivity and reduced skin vasodilation (Fortney *et al.*, 1981). The physiological mechanisms that mediate the decrease in sweat rates is due to both the singular and combined effects of plasma hypertonicity and hypovolemia. During exercise in the heat, hypohydration elicits hyperosmotic hypovolemia, with Sawka and colleagues (1985) reporting an increase in plasma osmolality and a decrease in plasma volume increasing with the severity of hypohydration. This response has been explained to be responsible to mediate the reduced sweating rates with increasing severity of hypohydration.

2.6.5.2. Cardiovascular and circulatory responses to hypohydration

One of the consequences of hypohydration is the increase in cardiovascular demand during exercise under heat stress (Casa *et al.*, 2000). As a result of hypohydration and in comparison to euhydration, plasma volume is reduced (hypovolemia) and there is an increase in viscosity (hypertonicity), both of which are influenced by the magnitude of hypohydration (Sawka *et al.*, 1992) which, may lead to a reduced venous return. Even though these physiological changes are present, the demand for blood flow to the skeletal muscle and skin still remains. While at the same time a hypohydrated individual attempts to maintain cardiac filling pressure by limiting peripheral circulation; however, during heat stress where the dissipation of heat is needed at the skin the cardiovascular

system is challenged and cardiac output declines. Through a reduction in cardiac filling (Stöhr *et al.*, 2011) and a decline in stroke volume (González-Alonso *et al.*, 1997), there is an inability to maintain cardiac output (Montain and Coyle, 1992) and muscle blood flow (González-Alonso, Calbet and Nielsen, 1998), which is exacerbated when hyperthermic (González-Alonso *et al.*, 1997; Sawka, Cheuvront and Kenefick, 2012). A reduced blood volume leads to a reduced stroke volume which therefore results in a compromise in cutaneous circulation (Nadel *et al.,* 1980). Along-side the reduced blood volume that leads to a reduced blood flow and ability to dissipate heat, there is a decrease in sweating (Strydom and Holdsworth, 1968). In an attempt to maintain circulatory demand, HR is increased to compensate for losses in blood volume from sweat production and for every % of water deficit HR has seen to be 4 $b.min^{-1}$ higher (Sawka *et al.,* 1984; 1985). In another study, González-Alonso *et al.* (1997) reported hypohydration to exacerbate the increase in HR - HR was reported to increase by 5 ± 1% when exercising at 72% $\dot{V}O_{2max}$ for 30 min and increased by 9 ± 1% when exercising with hyperthermia and ~4% hypohydration.

Hydration status can negatively influence exercise performance when exercising in hot and humid environmental conditions (Sawka *et al.*, 1992; Bardis *et al.*, 2013; James *et al.*, 2017). There have been a number of investigations that have shown a body mass loss of ≥2% can lead to an impairment in exercise capacity and performance due to an increase in thermoregulatory and cardiovascular strain experienced in these

conditions (Montain and Coyle, 1992; Gonzalez-Alonso *et al.*, 1995). However, it is well-appreciated that not only is hypohydration potentially problematic to exercise performance but also can be dangerous to health (Wyndham and Strydom, 1969). Conversely, when an individual over consumes fluid over a short period of time, they can dilute plasma sodium concentrations levels which can lead to hyponatraemia. Hyponatraemia is defined as a sodium concentration of less than 135 $mEq \cdot L^{-1}$, which can be fatal if not treated (Armstrong *et al.*, 1993). There are a number of risk factors as well as over consuming fluids, the fluid composition (e.g. plain water), low body mass, long exercising time, lack of experience, use of nonsteroidal anti-inflammatory drugs (NSAIDs) and female sex (Ayus, Varon and Arieff, 2000; Davis *et al.*, 2001; Hew *et al.*, 2003; Almond *et al.*, 2005).

2.6.6. Perceptual effects of hypohydration

Not only does exercise-induced hypohydration mediated physiological strain primarily driven by hypovolemia but also increases perceptual strain. Whereby, hypohydration stimulates an increase in perception of effort, thermal strain and sensations of thirst (Bardis *et al.*, 2013; Cheuvront and Kenefick, 2014; Logan-Sprenger *et al.*, 2015). The increase in extracellular osmolarity as highlighted earlier, stimulates the secretion of arginine vasopressin (AVP) an anti-diuretic hormone, which prevents water loss by decreasing kidney water excretion (Hew-Butler, 2010). In one experiment (held outdoors on a 12 km course performed in warm

environmental conditions (26.1 ºC to 26.3 °C)) Stearns *et al.* (2009) reported that RPE and TS were both greater when runners were hypohydrated. The methodology used by Stearns *et al.* (2009) to induce hypohydration was a restriction of fluid intake 22 h prior to the experimental trial. While unable to directly conclude as no exit interview was conducted, it is likely that participants were aware of their true hydration status. Potentially the lack of study blinding could have increasing perceptions of exertion, thermal discomfort and thirst when hypohydrated. However, the increased perceptual strain reported in this study demonstrated the interplay of these behaviours on exercise performance outcomes as an increase in RPE and TC linearly increased with a decline in running velocity. In other studies which attempted to control thirst sensation, hypohydration was shown to impair performance without an increase in thirst sensation (Adams *et al.*, 2018). Other perceptual data was not included and therefore the authors were unable to directly assess the influence of RPE and thermal perceptions on this performance outcome.

2.6.7. Summary of hypohydration

There is a large body of the evidence that has supported exercise capacity and performance in the heat to be impaired to a greater extent when hypohydrated (>2%) in laboratory-controlled studies due to an increase in physiological and perceptual strain experienced when exercising in these conditions, than compared to when exercising with hypohydration during

field based studies, in a euhydrated state or in less thermally stressful environmental conditions. Despite this, the methodologies used to induce hypohydration within some of these studies has raised a number of concerns on the potential influence this may have had on the performance outcomes documented. While unable to conclude to what extent this influence exactly had, it is known that the awareness of intervention received and, perception and expectation of treatment can have either a placebo or nocebo effect on performance. To date only two recent studies have successfully blinded participants of their true hydration status when hypohydrated. Both James *et al.* (2017) and Funnell *et al.* (2019) reported 15 min exercise performance to be impaired during the hypohydrated trial. However, yet to be investigated is the influence of perception and expectation of hydration status on exercise performance in the heat. **Therefore, in experimental study 1 presented in Chapter 3 of this thesis, the effect of perception and expectation of hydration status was investigated on cycling performance in the heat, when participants were hypohydrated in both experimental trials induced by exercise and blinded by the use of a nasogastric tube.**

2.7. Cooling Interventions

2.7.1. Introduction to cooling interventions

In addition to hydration interventions, a number of cooling interventions have been adopted in an attempt to alleviate thermoregulatory strain. The upcoming subsections within this literature review will primarily focus on

the application of external cooling, applied before exercise (pre-cooling) and during exercise (per-cooling) on subsequent exercise performance in the heat.

2.7.2. Whole body pre-cooling

The effect of pre-cooling on exercise capacity and performance has received the greatest amount of attention compared to per- and post-cooling interventions. A number of different pre-cooling methods have been investigated with the overall aim to lower an athlete's preliminary core temperature and feelings of thermal strain, and these methods include; whole-body cold-water immersion (CWI) (Booth, Marino and Ward, 1997; González-Alonso *et al.*, 1999; Kay, Taaffe and Marino, 1999), cold air exposure (Hessemer *et al.*, 1984; Dae Taek Lee and Haymes, 1995), cold water showering (Drust, Cable and Reilly, 2000), ice slushy/slurry (Ihsan *et al.*, 2010) and wearing cooling packs and/or ice vests (Martin *et al.*, 1998; Arngrímsson *et al.*, 2004). Different cooling techniques elicit different responses from the body, with some influencing the external shell only (e.g. ice vests, cool air), while others can influence both the internal and external shell (e.g. CWI, cold air exposure, or ice slurry). Early cooling research primarily was focused within military and workforce populations (e.g. mining). As within these settings it is difficult to dissipate heat due to exposure to hot conditions, protective clothing worn (i.e. military, fire fighter), and equipment needed to be carried (DeGroot *et al.*, 2013). Burton and Bazett (1936) were one of the first to examine the thermoregulatory

responses to immersion in water baths of varying temperatures (4 ºC to 38 °C) but it wasn't until the 1980s that cooling interventions started to receive attention for sporting performance (Schmidt and Bruck, 1981).

In a meta-analysis conducted by Bongers, Hopman and Eijsvogels (2017) (Figure 2.6) it was reported that a mixed combined pre-cooling approach is the most effective intervention to enhance exercise performance, by providing a more "aggressive" cooling stimulus to lower preliminary thermal strain. As cooling a larger surface area of the body provides a greater potential for heat exchange, than compared to cooling local body areas. After a mixed combined approach and in this order, CWI, cold water/ice slurry ingestion, cooling packs and ice vests were the most effective cooling techniques applied to enhance exercise performance.

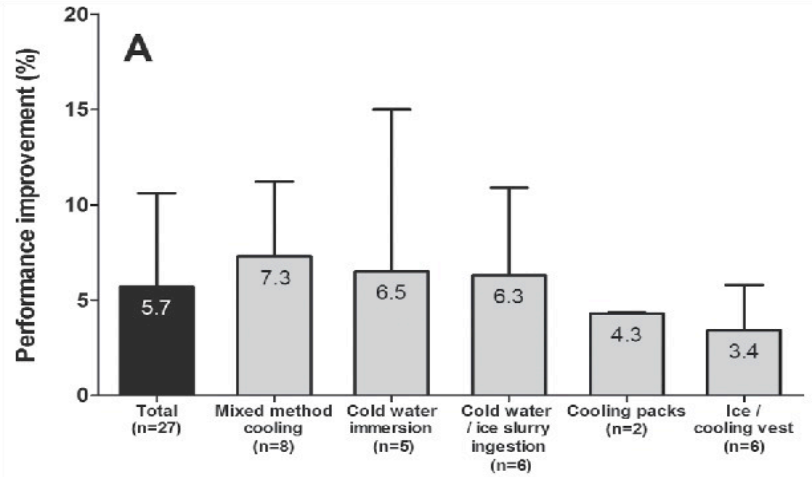

Figure 2.6. An overview of the average performance improvement and the beneficial effects of different precooling strategies (grey bars). Data are presented as mean ± SD. [Reproduced from Bongers *et al.* (2015), with permission].

The benefits of pre-cooling on exercise capacity and performance are likely multi-factorial with the primary mechanisms being a reduced thermoregulatory, cardiovascular and perceptual strain (Quod, Martin and Laursen, 2006) similar to that which occurs with acclimation (Nielsen *et al.*, 1993). One of the early documented applications of pre-cooling on sporting performance took place during the Atlanta Games in 1996, where the Australian Institute of Sport (AIS) issued cooling jackets for their athletes to wear prior to competition. On feedback, the athletes had reported that the cooling jackets had a positive contribution to their performance. Since this development, pre-cooling interventions have been investigated to reduce the physiological and perceptual strain experienced when exercising in the heat to mitigate the detrimental effects this has on performance (Martin *et al.*, 1998).

Pre-cooling using whole-body CWI is the main focus within this thesis and has been expressed as the "gold standard" method to lower core temperature (Casa *et al.*, 2007; Jones *et al.*, 2012). While there is no internationally accepted definition of "cold water" within this thesis it is defined as a water temperature below that is considered thermoneutral (<34 °C), in which individuals are unable to maintain deep core body temperature (Craig and Dvorak, 1966; Tipton and Bradford, 2014). Water has a thermal conductivity of ~25 times greater than that of air, allowing for heat to be lost via conduction to occur more rapidly during CWI than when exposed to the same air temperature (Smith and Hanna, 1975; Castellani and Young, 2016; Tipton *et al.*, 2017). Furthermore, water is

more dense than air, which consequently creates a greater pressure around the body, called hydrostatic pressure (Wilcock, Cronin and Hing, 2006). This pressure can cause intracellular fluid shifts and lead to a translocation of substrates from the muscle, increase cardiac output, reduce peripheral vascular resistance and increase the ability of the body to transport substrates (Wilcock, Cronin and Hing, 2006). The subsequent sections will discuss effects of CWI on exercise performance before reviewing the physiological and perceptual mechanism in response to CWI.

2.7.1. Pre-cooling on prolonged exercise performance

Within the pre-cooling literature there is large variability within the methodology used in terms of the exercise protocol applied (e.g. open or closed-loop design), the type of cooling intervention implemented, participant characteristics (e.g. trained, untrained) and environmental conditions used. Firstly, and in brief, the effectiveness of pre-cooling on single and repeated sprint performances have been equivocal (Brade, Dawson and Wallman, 2013). Studies investigating single sprint performances lasting between 5 s to 30 s have reported no performance benefits to occur from pre-cooling (Drust, Cable and Reilly, 2000; Cheung and Robinson, 2004; Duffield and Marino, 2007). While during repeated intermittent sprint performance studies a small positive effect size (d = 0.44) has been reported (Castle *et al.*, 2006, 2011; Duffield and Marino, 2007). In one study, Castle *et al.* (2006) found that peak power output was improved by 4% in the heat (34 °C, 52% rh) after pre-cooling with ice packs on the upper legs, compared to when no pre-cooling was applied.

With sprint performances highly influenced by the temperature of the muscle, contractile function and/or anaerobic metabolism (Asmussen and Bøje, 1945; Faulkner *et al.*, 2013), cool muscles do not only reduce anaerobic metabolism but also impair voluntary contraction, resulting in a reduced power output (Bigland-Ritchie *et al.*, 1992).

Therefore, the effects of pre-cooling have appeared to only be beneficial to exercise performance when thermal strain and thermal load is high (Duffield and Marino, 2007). In an earlier meta-analysis, Bongers *et al.* (2015) reported that when ambient air temperatures exceeded 30 °C, pre-cooling interventions can improve exercise performance in the heat by 5.7 ± 0.9%. In support of Duffield and Marino's (2007) earlier findings, Faulkner *et al.* (2019) recently investigated the ambient air temperature threshold (24 ºC, 27 ºC & 35 °C) at which pre-cooling became an effective intervention for enhancing endurance performance. Cycling TT performance was faster following pre-cooling in both 35 °C (6.2%) and 27 °C (2.6%) but not when exposed to 24 °C.

As shown earlier in Figure 2.6. after mixed cooling methods, CWI is the second most effective cooling intervention to offset the detrimental effects of exercising in the heat. To date, there is moderate evidence on the application of CWI to improve exercise performance in the heat (Jones *et al.,* 2012). **Therefore, CWI is the main pre-cooling intervention focused on within this thesis, whereby pre-cooling via CWI was investigated on cycling performance in Chapter 4.** Current evidence

shows pre-cooling via CWI increases exercise capacity (González-Alonso *et al.*, 1999; Hasegawa *et al.*, 2006; Siegel *et al.*, 2012) distance covered (Booth, Marino and Ward, 1997; Kay, Taaffe and Marino, 1999) and power output (Duffield *et al.*, 2010) during exercise performance tests in the heat. In a well-known study by González-Alonso *et al.* (1999), the influence of core temperature on the development of fatigue was investigated. It was demonstrated that sub-maximal cycling duration was inversely related to starting core temperature, where pre-cooling lowered initial core temperature to 35.9 ± 0.2 °C, enhancing individuals' ability to continue exercising by ~37%. In contrast, pre-warming individuals to 38.2 ± 0.1 °C decreased exercise duration by ~39%.

In an exercise performance study, Booth, Marino and Ward (1997) instructed participants to cover as much distance as they possibly could during a 30 min running TT in a hot environmental condition (31.6 °C, 60% rh) after ~60 min of CWI (23 ºC to 24 °C). Upon exiting CWI, core temperature was lowered by ~0.7 °C, reducing thermoregulatory strain at the onset and throughout exercise. This reduced thermoregulatory strain was observed to benefit exercise performance as participants covered 4% greater distance (304 ± 166 m) after pre-cooling than compared to non-pre-cooling trial. The authors had reported that when no pre-cooling was applied, participants maintained a set running pace from the onset, compared to pre-cooling trial, where participants were able to run at a higher running velocity throughout and had the ability to increase speed near the end of the 30 min TT. Within the same research group, Kay,

Taaffe and Marino (1999) used a similar pre-cooling protocol to Booth, Marino and Ward (1997) prior to completing a 30 min cycling TT. The authors reported cycling performance was improved by 6% (~0.9 km) alongside reductions in skin temperature, but in the absence of a reduced core temperature. The authors noted a similar response in pacing, whereby the participants were able to increase power output towards the end of the TT. Furthermore, Duffield *et al.* (2010) investigated the effects of cooling the lower limbs in 14 ± 0.3 °C for 20 min before completing a 5 min warm-up and then a 40 min cycling TT in the heat (33 °C). After pre-cooling mean power output was significantly increased (198 ± 25 W vs. 178 ± 26 W), with the differences in mean power output occurring during the last 10 min of the TT, which is when the greatest physiological and perceptual strain was experienced. Core temperature was not different between trials at the onset of exercise; however, after the pre-cooling intervention the rate of rise in core temperature during the first half of the TT was blunted compared to non-pre-cooling trials.

Pre-cooling via CWI appears to be beneficial at lowering thermoregulatory and cardiovascular strain. Lowering an athlete's starting core temperature increases their heat storage capacity, enabling the athletes to maintain exercise intensity for an extended duration, prolonging the time taken to attain a "critical" core temperature (~40 °C) (Stannard *et al.*, 2011). This ultimately results in delaying the detrimental effects of hyperthermia-induced decrements and therefore offsets reductions in exercise performance occurring (Bongers *et al.*, 2015). While CWI is referred to as

the “gold standard” method to use, it is important to highlight that there are limitations with using CWI within a field setting, as it may be impractical to use before competition due to the nature of the sport, costs, transportation and discomfort to the athlete (Marino, 2002; Quod, Martin and Laursen, 2006; Eijsvogels *et al.*, 2014). Below the physiological and perceptual responses to pre-cooling specifically CWI immersion will be reviewed.

2.7.2. Physiological effects of pre-cooling via cold water immersion

2.7.2.1. Thermoregulatory responses to cold water immersion

In addition to the potential risk of cold-shock response upon acute immersion in cold water, one of the immediate physiological responses to occur is the vasomotor response, where a drop in skin temperature is followed by peripheral vasoconstriction and reduction in skin blood flow (Castellani and Young, 2016). This is an important physiological response that directs blood flow away from the periphery towards the core, to reduce convective heat transfer between the core and the skin, and therefore, increases insulation (Rowell, 1974; Stocks *et al.*, 2004; Castellani and Young, 2016). Initially, this response reduces heat loss; however, when water temperature falls below the thermoneutral temperature (33 ºC to 34 °C), vasoconstriction is insufficient to maintain core temperature, which leads to a decrease in core temperature. Despite initial increases in core

temperature, core temperature has been shown to be lower at the onset of exercise in some (Booth, Marino and Ward, 1997; Booth *et al.*, 2001; Duffield and Marino, 2007) but not all CWI studies (Kay, Taaffe and Marino, 1999).

It is evident that the greater the duration and extent of cooling stimulus applied, the greater the reduction in core, skin and muscle temperature reported (Yanagisawa *et al.*, 2007). Cold-water immersion protocols used before exercise and lasting for 60 min have reported to reduce core temperature by 0.7 ºC to 0.8 °C (Booth, Marino and Ward, 1997; Booth *et al.,* 2001). In comparison to the longer duration CWI protocols, Castle *et al.* (2006) immersed participants up to shoulder height for 20 min, with a much colder water temperature set at 17.8 ± 2.2 °C than compared to the water temperature used by Booth, Marino and Ward (1997) and Booth *et al.* (2001). Core temperature was reduced by 0.3 ± 0.3 °C but it did not reach the same magnitude of reduction that was reported during longer immersion periods (Booth, Marino and Ward, 1997; Booth *et al.*, 2001) likely due to the reduced length of time that participants spent immersed.

Depending on the cooling method applied, especially when more extreme temperatures are used, a time lag in core temperature reductions has been reported and this is known as an “after drop” effect (Romet, 1988; Booth, Marino and Ward, 1997). The after drop effect is a known phenomenon observed after immersion due to the conductive and convective heat transfer from the periphery to the core (Romet, 1988). A potential

explanation for why not all CWI investigations reporting core temperature to decrease during immersion (Bergh and Ekblom, 1979; Kay, Taaffe and Marino, 1999; Siegel *et al.*, 2012) but have found core temperature to decrease during the exercise bout (Siegel *et al.*, 2012) and still report improvements in exercise capacity or performance outcomes. Furthermore, the studies that reported no difference in core body temperature but found performance to improve, highlight that cooling the skin before the onset of exercise is an important regulator for exercise intensity. As mentioned earlier, Schlader *et al.* (2011a) examined the role of skin temperature on self-selected exercise intensity and reported that thermal perceptions associated with skin temperature were found to be important inputs into the selection of exercise intensity. In addition, Kay, Taaffe and Marino (1999) reported skin temperature was reduced by 5 ºC to 6 ºC without changes in rectal temperature and reported that lowering the skin temperature independently of rectal temperature improved 30 min exercise performance in the heat (31 °C).

2.7.2.2. Cardiovascular response to pre-cooling

Highlighted and expanded in detail earlier (2.4.1.3), cardiovascular strain during prolonged exercise in the heat has been proposed as an influential factor in limiting exercise capacity and performance in the heat (Coyle and González-Alonso, 2001; Cheung and Sleivert, 2004; González-alonso, Crandall and Johnson, 2008; Crandall and González-Alonso, 2010). As a result of a greater thermoregulatory burden during exercise in the heat

resulting in an increased skin blood flow demand, a compensatory progressive rise in HR and decline in stroke volume occurs (Rowell, Brengelmann and Murray, 1969; Trinity *et al.*, 2010; Lee *et al.*, 2015). With both high core and skin temperatures contributing to this phenomenon, pre-cooling investigations that lower core and skin temperature in isolation and in combination have been explored. Mixed evidence exists on the effects of pre-cooling on the HR response, with some showing HR to be unaffected by pre-cooling (Hessemer *et al.*, 1984; Kay, Taaffe and Marino, 1999; Drust, Cable and Reilly, 2000; Duffield *et al.*, 2003) while others have shown HR to be reduced following pre-cooling intervention (Lee and Haymes, 1995; Arngrímsson *et al.*, 2004; Cheung and Robinson, 2004; Castle *et al.*, 2006). Castle *et al.* (2006) reported HR was lower for the first 16 min of a 40 min cycling sprint protocol after a 20 min whole-body CWI (17.8 ± 2.1 ºC) compared to when pre-cooling was induced by cooling packs or a cooling vest. Similar to this finding, Duffield and Marino (2007) also reported that pre-cooling via CWI was effective at reducing HR during the first 10 min of exercise protocol, while the application of a cooling vest had no effect on HR response. These findings and others show that CWI is effective at lowering HR during the early stages of exercise; however, this response is short-lived and therefore, pre-cooling doesn't appear to be effective on cardiovascular strain during prolonged exercise (Dae Taek Lee and Haymes, 1995; Booth *et al.*, 2001; Arngrímsson *et al.*, 2004; Castle *et al.*, 2006; Duffield and Marino, 2007).

2.7.3. Perceptual effects of pre-cooling

As highlighted before (see section 2.4.2), the decrement in exercise performance has also been suggested as a conscious sensation that governs exercise intensity/termination and accelerated in the heat. Therefore, the lowering of physiological strain (i.e. lower core and skin temperature) after CWI would in theory alleviate perceived strain during exercise; however, while CWI has been well-documented to be highly effective at altering the physiological state of the individual, the evidence to support a beneficial effect on reducing perceived exertion is uncertain. In one study, the effects of CWI (23.8 ºC to 28.8 ºC) were investigated without the inclusion of exercise post immersion. The authors reported that 60 min of CWI lowered ratings of TC from feeling "comfortable" to reaching the mid-point between "too cool" and "much to cool" with changes in skin temperature but without changes in rectal temperature, as reductions were not reported until 3 min post-immersion (Marino and Booth, 1998). During exercise tests, CWI has been reported to be successful at reducing physiological strain but lower perceived exertion and thermoregulatory behaviours associated with exercising in the heat have not been found during exercise in most studies (Booth, Marino and Ward, 1997; Kay, Taaffe and Marino, 1999; Duffield and Marino, 2007). This outcome is likely explained by thermal behaviours being associated with a direct application of cooling and therefore, once cooling intervention has stopped the reduction in perceived thermal strain observed during pre-cooling intervention is not observed. Furthermore, the differences in perceptual responses are likely

due to the varying cooling methods used within these studies and the different exercise protocols (i.e. TT, TTE), exercise durations, intensities, environmental conditions and degrees of cooling (Wilson *et al.*, 2002).

2.7.4. Summary of pre-cooling via CWI

Cold-water immersion is shown to be a successful intervention in reducing an individual's preliminary core temperature and therefore reducing thermoregulatory strain before the onset of exercise and delaying the detrimental effects associated with hyperthermia-induced decrements in exercise performance. One of the reported limitations of pre-cooling, is that its capacity to alleviate thermal strain is short-lived during exercise (<25 min) (Marino, 2002). Therefore, in an attempt to prolong the benefits of pre-cooling, there is growing interest into investigating per-cooling in isolation and in combination with pre-cooling (Bongers *et al.*, 2015) and will be discussed in the upcoming subsections.

2.7.5. Introduction to per-cooling

During exercise, cooling (per-cooling) can be applied in an attempt to attenuate the rate of rise in core temperature and to reduce perceived thermoregulatory strain experienced when exercising in the heat (section 2.3.2.). Thermal strain is at its highest during exercise due to the increase in metabolic heat production from the exercising muscles. To date, the literature has primarily focused on the interventions to lower initial core temperature prior to exercise. However, there is emerging per-cooling

interventions that have been explored, which include; neck cooling collar (Tyler, Wild and Sunderland, 2010), menthol (Stevens *et al.*, 2017), face fanning, facial water spray (Stevens *et al.*, 2017), ice slurry/cold water ingestion (Lee, Shirreffs and Maughan, 2008; Schulze *et al.*, 2015) and the application of ice vests and/or ice jackets (Eijsvogels *et al.*, 2014).

These cooling methods can be applied continuously or intermittently during exercise, which have both resulted in beneficial physiological effects in some (Ruddock *et al.*, 2017) but no differences in others (Tyler and Sunderland, 2011). The type of per-cooling intervention applied has been demonstrated to influence the impact on exercise performance as reported in an meta-analysis by Bongers *et al.* (2015). It was highlighted that wearing a cooling vest was more effective on exercise performance (+21.5%), than ingesting cold water (+11%) or applying cooling packs (+8.4%) during exercise. The success of a cooling intervention on exercise performance appears to be dependent on the magnitude of surface area cooled and therefore, the efficacy of cooling stimulus (Bongers *et al.*, 2015). The larger surface area cooled may contribute to reducing thermal strain and therefore subsequently improve exercise performance (Hasegawa *et al.*, 2005).

It is not uncommon to report that per-cooling has no direct effect on the thermoregulatory or cardiovascular systems, which are seemingly unchanged (Cheung, 2010; Ruddock *et al.*, 2017; Best *et al.*, 2018) and will be reviewed in the upcoming subsections. Even though reductions in

physiological strain are not commonly observed, reductions in perceived thermal strain have been reported and discussed in detail in section 2.4.2. Considerations should be taken when investigating per-cooling interventions, as each have practical limitations, which are dependent on sport and level of contact allowed with athlete during exercise bout. While the application of cooling vests has proven to be a successful intervention in enhancing exercise performance, the practically of this method used during cycling or running events in field settings potentially may be detrimental to an athlete's performance, rather than improve it, due to the weight of vest. Therefore, the type of per-cooling intervention investigated is the application of a neck cooling collar. **Per-cooling via the application of neck cooling collar will be the main focus within this review and investigated in Chapter 4.**

2.7.5.1. Introduction to cooling the head and neck

Recent and indirect evidence offers uncertainty that humans do selective cool the brain to ensure that brain temperature does not exceed core temperature (Mariak *et al.*, 1994; Cabanac, 1998). Different surface area regions of in the body receive temperature stimuli in different ways, which induces the thermal sensation response (Kenshalo, Decker and Hamilton, 1967; Stevens, Marks and Simonson, 1974). Physiological evidence from studies that have systematically investigated different body regions (i.e. forehead, check, forearm, thigh, calf) have shown thermal sensitivity varies across the body, with the face being the most sensitive region to

warmth compared to other upper and lower extremities (Stevens, Marks and Simonson, 1974; Stevens and Choo, 1998; Nakamura *et al.*, 2008), therefore, providing the greatest alliesthesial sensitivity (Cotter and Taylor, 2005). With this understanding, interventions to offset the detrimental effects to exercise performance in the heat and lower the temperature of the head, face and neck to reduce physiological and perceptual strain are reviewed in the upcoming sections. Per-cooling has received far less attention than pre-cooling, although there has been recent interest due to advances in practicality of cooling devices where an ice vest jacket or a neck collar can be applied quickly without causing too much disruption to the athlete during competition. Even though there is emerging evidence of how the application of cooling effects exercise performance, there are limited data on the effects on endurance performance and on the athletic population (Kay, Taaffe and Marino, 1999).

2.7.5.2. The effect of neck and head cooling on exercise capacity and performance

Limited research has been conducted in exploring the effects of neck and head cooling on exercise capacity and performance in the heat. Facial water spraying at 30 s intervals was reported by Ansley *et al.* (2008) to increase TTE by 21 min (51%), when nine males cycled at 75% of $\dot{V}O_{2max}$ in 29 °C (50% to 70% rh). In one of the earlier studies, Palmer, Sleivert and Cotter (2001) used a water-perfused garment to cool the head and neck

region by circulating 1 °C cold water at a rate of 1.1 L·min^{-1} during a 30 min submaximal (60% $\dot{V}O_{2max}$) treadmill exercise before completing a 15 min TT in the heat (33 °C). During the per-cooling trial the distance covered in the TT was improved by 3.3 ± 3.4%, compared to when no per-cooling was applied.

Tyler and Sunderland (2011a) reported that endurance runners were able to run 13.5 ± 3.8% longer when wearing a neck cooling collar during a TTE test in a hot environmental condition. From the same laboratory the authors conducted a similar study design that investigated 15 min TT running performance with neck cooling collar (Tyler and Sunderland, 2011b). Improvements in performance outcomes were reported to occur without changing the physiological state of the individuals, supporting the central governor hypothesis. It has been proposed that cooling the neck and head may send a false signal of the actual thermal state of the individual back to the brain. This perceived false thermal state may influence the athlete's behaviour by self-selecting a faster pace during exercise leading to an improvement in performance compared to when exercising without a cooling collar. Caution has been expressed throughout the literature though because a perceived false signal indicating lower thermal status than the actual physiological state, may increase an individual's risk of developing heat-related illness (Bongers, Hopman and Eijsvogels, 2017). Tyler and Sunderland (2011a) expressed that effective monitoring and briefing to the individual is advised, as potentially masking thermal strain may in theory allow the individual to tolerate a higher core

temperature and override the thermal signals that govern the termination of exercise. That said, exercise has been shown to be terminated with the same level of perceived TC and RPE in a hot environment (Tyler and Sunderland, 2011a).

2.7.6. Physiological responses to per-cooling the head and neck

2.7.6.1. Thermoregulatory responses to cooling the head and neck

The effectiveness of cooling the head and neck on altering core temperature relies upon the method of cooling used and the thermal stress encountered; however, it is often reported that cooling the head and neck region is unable to lower core temperature (Tyler and Sunderland, 2008; Bongers *et al.*, 2015; Tyler, Sunderland and Cheung, 2015). This is likely due to the small surface area that cooling was applied to and potentially the cooling method was ineffective for the transfer of heat (Ruddock *et al.*, 2017). The head and neck comprise only ~10% of the total body surface area (Du Bois and Du Bois, 1989) whereas cooling a larger surface area such as the torso (~36% of the body's surface area) can lower skin temperature and increases the core to skin radiant, allowing for heat to be exchanged and lowering thermal strain. However, there are some disadvantages to cooling such a large surface area with these cooling techniques (i.e. ice vests), which include weight and accessibility during competition. Neurophysiological evidence has shown a higher density of thermal afferents arising from the face, where Crawshaw *et al.* (1975)

found that cooling the forehead was more than three times as effective (per size of the area) in decreasing ongoing sweating as cooling the lower leg region. This is due to the face region having a high concentration of thermoreceptors and is regarded as the most thermally sensitive region in the body (Cotter and Taylor, 2005). Therefore, cooling this area may offer powerful and beneficial responses to the autonomic and behavioural systems during exercise under heat stress.

2.7.7. Perceptual responses to per-cooling the head and neck

The impairment in exercise capacity and performance in the heat is also mediated by a central component, driven by information feed-back from the central and peripheral receptors (e.g. thermoreceptors, chemoreceptors, baroreceptors) regarding the state of the body. Therefore, manipulating the perceptual state of how an individual feels without alterations in physiological state (i.e. reduce thermoregulatory or cardiovascular strain) has been shown to improve exercise capacity and performance in the heat (Tyler and Sunderland, 2008). At first, the improvement in performance reported was proposed to be due to a reduced heat storage; however, this was soon dismissed. McCaffrey *et al.* (1975) expressed that the improved performance was due to the cooling intervention, which directly cooled the blood in the jugular vein, which flows into the carotid artery. Cooling the neck fails to alter core temperature, likely due to the small surface area cooling is applied to. However, as introduced earlier (2.7.5.1) this area has a high thermal

sensitivity due to greater density of cold-sensitive thermal afferents in this area and potentially cooling the neck may provide an ergogenic effect (Cotter and Taylor, 2005). Therefore, it is unsurprising that the temperature of the head has been shown to be highly influential on TC (Kato *et al.*, 2001; Nakamura *et al.*, 2008). Cooling the head and neck region has shown to reduce perceived exertion and thermal sensations/discomfort during exercise in a hot environment, permitting an increase in self-selected work by false signal of thermal strain (Cheung, 2010; Stevens, Taylor and Dascombe, 2017).

2.7.8. Summary of pre and per-cooling interventions for exercise in the heat

It is well-accepted that exercise capacity is limited and performance is impaired when exposed to high thermal loads from the environment, due to marked increase in physiological and perceptual strain experienced in these conditions (see section 2.4). The use of cooling interventions to offset the detrimental effects of hyperthermia-induced decrements in exercise performance warrants further investigation. As it currently stands, pre-cooling via CWI is the most effective approach at reducing core body temperature before the start of exercise, by increasing an individual's capacity to store more heat to delay the attainment of a high core temperature. On the other hand, per-cooling during exercise when thermal strain is at its highest, seemingly offers a more efficient heat loss avenue, attenuating the rate of rise in core temperature, reducing perceived thermal

strain and subsequently offsetting performance impairments. Both pre- and per-cooling interventions when used in isolation have evidence to support that exercise capacity and performance is improved compared to control group. However, far less is known on the effectiveness of combining both pre- and per-cooling on exercise performance, with attention primarily drawn to the use of internal cooling interventions. To date, combined internal pre- and per-cooling interventions have not resulted in a cumulative benefit to exercise performance by increasing the margin to store heat and reducing thermal strain during exercise. **Therefore, the purpose of experimental study 2 presented in Chapter 4 of this thesis was to investigate the effects of external pre- and per-cooling used in isolation and in combination on cycling performance in the heat (40 ºC) in highly trained individuals.**

2.8. Heat acclimation (HA)

2.8.1. Introduction to heat acclimation

Repeated exposure to a thermal stress from the environment (i.e. temperature, relative humidity, solar radiation, air velocity), with metabolic heat production from exercise (i.e. active) or without (i.e. passive) and in some cases type of clothing worn during exposure that impedes heat loss (i.e. combat), can induce beneficial thermal adaptations to improve exercise capacity (Nielsen *et al.*, 1993) and exercise performance (Lorenzo *et al.*, 2010) in the heat. This extended tolerance is achieved through a number of behavioural, morphological and

physiological adaptations, which result from internal homeostasis being repeatedly challenged (Taylor, 2014; Périard, Racinais and Sawka, 2015). In the following sections, the primary adaptations to HA are discussed and therefore in brief, include; thermoregulatory, cardiovascular, fluid-electrolyte, metabolic and thermal perceptions. The thermoregulatory adaptations are a reduced core temperature at rest (Buono, Heaney and Canine, 1998; Kampmann *et al.*, 2008; Daanen *et al.*, 2011) and during exercise and an increase in sweating sensitivity and rate (Lorenzo and Minson, 2010; Buono *et al.*, 2018). Cardiovascular adaptations are demonstrated by an improved cardiovascular stability (Frank *et al.*, 2001; Périard *et al.*, 2016) by a lowering of HR, an increase in stroke volume, and a better maintained cardiac output (Nielsen *et al.*, 1993). Thermal perceptual adaptations are evidenced by a lower RPE, improved TC and TS while metabolic adaptations are shown by an improved exercise economy (Tyler *et al.*, 2016).

There are two pathways an individual can take to induce thermal adaptations. Acclimatisation is when the adaptations occur when exposed to a natural environment, this can happen during a training camp or time spent in hot/moderate climate condition similar to what is to be expected during competition (Corbett *et al.*, 2014). The other main focus of this thesis is the process of acclimation, whereby, an individual is repeatedly exposed to an artificially controlled environment, typically seen in an environmental chamber. Within this artificial environment ambient air

temperature and relative humidity can be manipulated and individuals can be actively or be passively heated (Sawka *et al.*, 2011).

2.8.1. Induction of heat acclimation

The environmental conditions should mimic the environmental conditions (i.e. temperature & humidity) that competition is held in to gain the thermal adaptations required to benefit performance (Racinais *et al.*, 2015). Typically, the greatest physiological and perceptual strains are observed at the start of the HA protocol with gradual reductions in these respective strains reported thereafter. For both coaches and athletes it is important to consider the limitations and barriers of undertaking a HA protocol. To benefit from the adaptations it should be integrated into an athletes training schedule as close to competition as possible, because the adaptations are short lived. Investigations into the rate of decay have been explored. In one study, Daanen *et al.* (2011) observed that for every day spent without exposure, adaptations were lost at approximately 2.5% per day, after participants underwent nine days of HA. It has been widely accepted that the adaptations to occur first, which are circulatory adjustments, are the first to decay (Pandolf, Burse and Goldman, 1977; Pandolf, 1998; Saat *et al.*, 2005; Garrett *et al.*, 2009). The majority of HA literature has focused on the induction rather than the rate of decay of acclimation, with many confounding methodological issues to be considered including; aerobic fitness level, heat exposure duration, heat exposure frequency and environmental conditions. Some of these

methodological examples offer inconsistent data and further research is warranted.

It is important to take into consideration that this is a crucial phase within an athletes training schedule, where an athlete will be tapering with the emphasis placed upon recovery by reducing training volume and training stress (Bosquet *et al.,* 2007; Mujika, 2010). An overly exerting HA protocol may compromise subsequent exercise performance as a result of an increase in the stress response, where the activation of the hypothalamus-pituitary-thyroid axis (HPA) leads to an increase in cortisol secretion (Wright, Selkirk and McLellan, 2010; Daanen, Racinais and Périard, 2018; Reeve *et al.*, 2019). Furthermore, accessibility, feasibility and time are major considerations that need to be planned out.

Individuals with a high aerobic fitness level have been shown to adapt more rapidly compared to untrained individuals (Pandolf *et al*., 1977; Cheung and McLellan, 1998), with core temperature plateauing around day six of HA with individuals that have above average $\dot{V}O_{2max}$ levels. However, larger decreases in HR and core temperature from pre-acclimation baseline measurements have been reported in individuals with low to moderate aerobic fitness level. Therefore, individuals with a lower $\dot{V}O_{2max}$ may experience a greater benefit from a period of HA (Cardarette *et al.,* 1988; Shartz *et al.,* 1977; Cheung and McLellan, 1998). Cheung and McLellan (1998b) investigated 14 days of HA (1 h treadmill exercise at 40 °C and 30% rh) in moderately (<50 $mL \cdot kg^{-1} \cdot min^{-1}$) and highly fit (>55

$mL \cdot kg^{-1} \cdot min^{-1}$) males. Individuals with a higher fitness level were reported to have a greater improvement in physiological strain and the authors expressed that these benefits were likely due to fitter individuals able to tolerate a higher rectal temperature as voluntarily termination of exercise occurred at an ethical limit of 39.14 ± 0.21 °C, compared to the moderately fit individuals who became exhausted below this threshold (38.79 ± 0.31 °C).

2.8.2. Heat acclimation protocols

2.8.2.1. Frequency of heat acclimation

Typically, HA protocols are divided into three main time course durations; short-term (STHA) lasting ≤7 days, medium-term (MTHA) lasting between 7 days to 14 days and long-term (LTHA) lasting greater than ≥14 days (Garrett, Rehrer and Patterson, 2011). STHA and MTHA protocols (5 days to 14 days) have received the majority of attention compared to LTHA protocols (Tyler *et al.*, 2016), with HA protocols lasting 4 days to 6 days conferring up to 80% of the adaptations reported to occur during a LTHA protocol (Armstrong and Maresh, 1991; Pandolf, 1998). It is well-accepted that the time-course of these adaptations vary, depending on the variable, with the magnitude of these adaptations dependent on the duration of exposure to heat stress (Fox *et al.*, 1963, 1964; Garrett, Rehrer and Patterson, 2011). For example, changes in the cardiovascular system such as a lowering in HR typically occur rapidly within the first three days, well before improvements are observed in sudomotor function and

exercise performance (Armstrong and Maresh, 1991; Périard *et al.*, 2016). Short-term protocols seem to be more convenient for athletes to integrate into training/tapering programs, where training volume and training stress are already high and cannot be further compromised by a high volume of thermal strain (Mujika *et al.*, 2004; Zurawlew *et al.*, 2016).

2.8.2.2. Specificity of exercise mode during heat acclimation

The mode of exercise used to induce the adaptations does not seem to interfere with the physiological adaptations from HA, as long as core temperature is elevated to at least ~38.5 °C (Taylor *et al.*, 1997; Garrett, Rehrer and Patterson, 2011). However, it is advised that individuals should use the exercise mode that they will be performing (Taylor and Cotter, 2006), while exercising in the similar environmental conditions expected during competition to achieve optimal adaptation. Different HA induction methods have been explored, yet the optimal HA protocol is still unknown and open for debate, despite a large body of research manipulating the intensity and duration of exercise, the frequency of HA exposures and the type of HA used (Taylor, 2014).

2.8.2.2.1. <u>Self-regulated and constant work rate protocols</u>

During self-regulated HA protocols the individual self-selects a workload according to their fitness ability or are instructed to exercise at a set RPE (Armstrong *et al.*, 1986; Cheung, 2010; Garrett, Rehrer and Patterson,

2011). This method was first adopted by the U.S marines to reduce the number of heat casualties during long periods aboard ships (Armstrong *et al.*, 1986). A limitation to this approach within a research setting is that it does not provide clear and concise data, making it difficult to interpret the thermal adaptations taking place. Furthermore, individuals will likely have variations in workload over the course of exposure days and among each other, making it difficult to determine within and between participant differences on physiological and perceptual response during HA exposure. Historically, constant work rate exercise protocols (also known as fixed intensity exercise protocols) were the most common HA approach utilised. In this format, the work prescribed during the HA sessions are determined from baseline pre-acclimation measurements and remain set for the duration of the HA protocol (Taylor, 2000), whereby individuals exercise at a fixed intensity (i.e. % of $\dot{V}O_{2max}$). Typically, at the start of the HA protocol the highest thermal strain will be experienced and as thermal adaptations develop potentially the optimal thermal stimuli may not be achieved later on during the protocol (Taylor and Cotter, 2006; Taylor, 2014; Gibson *et al.*, 2015).

2.8.2.2.2. <u>*Isothermic HA protocols*</u>

Elevations in both skin and core temperature are a fundamental requirement to induce thermal adaptations (Fox *et al.*, 1963a; Regan, Macfarlane and Taylor, 1996) and without a sufficient increase in rectal body temperature defined as thermal impulse in this thesis, adaptations

will not occur (Taylor, 2014; Tyler *et al.*, 2016; Reeve *et al.*, 2019). An isothermic method requires the individual to exercise to attain a target rectal temperature (e.g. 38.5 ºC or 39.0 °C) or a set increase in rectal temperature (e.g. +1 °C), once reached it is maintained using passive and active heat stress. This ensures that equal thermal strain is attained throughout the duration of the HA protocol (Taylor and Cotter, 2006; Garrett *et al.*, 2014; Gibson *et al.*, 2015).

Fox *et al.* (1961; 1963a) was one of the first to investigate the effects of a 1 °C increase in core temperature on adaptations using passive methods. The authors suggested that an increase in core temperature was the primary input for thermal adaptations to take place. However, while some thermal adaptations were observed, the thermal strain reduced as participants acclimated. Therefore, thermal strain was not equal across all experimental trials and potentially insufficient to offer complete adaptation to the heat. Following on from Fox *et al.* (1963a), Regan, Macfarlane and Taylor (1996) investigated the role of elevated skin temperature with the attainment of a 1 °C increase in rectal temperature on thermal adaptations. Through the manipulation of ambient air temperature, participants either exercised in temperate (22.4 °C) or hot (38 °C) conditions to manipulate skin temperature. The increase in skin temperature with an increase in rectal temperature was observed to be a key mechanism in inducing thermal adaptations, rather than an increase in rectal temperature only.

Therefore, for thermal adaptations to occur, both rectal and skin temperatures must be elevated above the sweating threshold. There is evidence to support that implementing a isothermic approach using a target rectal temperature (38.5 °C), offers a more complete adaptive potential (Garrett, Rehrer and Patterson, 2011; Garrett *et al.*, 2012, 2014; Gibson *et al.*, 2015; Tyler *et al.*, 2016), than attaining an absolute increase in rectal temperature (Fox *et al.*, 1963a; Regan, Macfarlane and Taylor, 1996). In one study, Gibson *et al.* (2015) compared the adaptive responses between a fixed intensity, isothermic (target rectal temperature ~38.5 °C) and a isothermic progressive (target rectal temperature ~39 °C) HA method over a short-term (five days) and medium-term (ten days) time-course in recreationally active participants, where HA sessions lasted for 90 min per day. The authors observed no differences in magnitude of adaptations when comparing an isothermic HA protocol to a fixed or isothermic progressive HA method. Another main finding was that short-term HA provided a sufficient thermal stimulus to induce adaptations with no further enhancement reported after medium-term HA. A short-term isothermic protocol has also been investigated in trained individuals, where typically they have already attained some thermal adaptations from training exposure. Garrett *et al.* (2014) investigated an isothermic HA protocol with dehydration and reported that five days of HA induced effective adaptations, with more pronounced changes from permissive dehydration.

The research thus far surrounding isothermic HA has brought about a fundamental question on what thermal stimulus is enough and how long is required to be at this threshold to induce the physiological and perceptual adaptations. Garrett *et al.* (2014) had a group of participants cycle for 90 min at a fixed isothermic temperature (target rectal temperature ~38.5 °C) for five days in a hot environment (40 °C, 60% rh). A heat stress test (HST) was conducted pre- and post- the HA period, where participants cycled for 60 min in a hot environment (35 °C, 60% rh) at 50% $\dot{V}O_{2max}$. Short-term HA was found to induce favourable adaptations, including an increase in plasma volume and a reduced rectal temperature at the end of exercise by -0.3 °C. **Evidence to date has shown the beneficial effects of following a STHA and MTHA isothermic protocol lasting for 90 min per day; however, yet to be investigated is if these adaptations can occur with a shorter exposure duration per day. Therefore, in Chapter 5 of this thesis, the effect of a 60 min daily isothermic HA protocol (target rectal temperature ~38.5 ºC) on the physiological and perceptual adaptations is investigated, further comparing if there is a time course response to these adaptations over a short-term (five days) and medium-term (ten days) period.**

2.8.3. Physiological adaptation to heat acclimation

2.8.3.1. Thermoregulatory responses to heat acclimation

The primary thermoregulatory adaptation to occur from HA is a reduction in core temperature at rest (~0.3 ºC to 0.5 °C) (Fox *et al.*, 1967; Shvartz *et*

al., 1979; Buono, Heaney and Canine, 1998; Patterson, Stocks and Taylor, 2004), with the greatest reduction reported to be -0.6 ºC to 1.0 °C, after intermittent and consecutive days of heat exposure (Gill and Sleivert, 2001). Differences between sexes have also been documented, where males have demonstrated a reduction in rectal temperature at rest after STHA, whereas females required a further five days (MTHA) to elicit the similar thermoregulatory responses (Mee *et al.*, 2015). In a recent meta-analysis, Tyler *et al.* (2016) reported a moderate-to-large effect size for HA on reducing core temperature at rest (-0.18 ± 0.14 °C), overall mean (-0.31 ± 0.31 °C) and comparable time-points during exercise (-0.34 ± 0.24 °C). A number of different core temperature measurement sites were included in the meta-analysis. Resting measurements were comparable across the different measuring sites although there was a greater effect size for oesophageal and gastrointestinal (rectal: -0.18 ± 0.15 °C, oesophageal: -0.22 ± 0.09 °C, tympanic: -0.10 ± 0.10 °C; gastrointestinal: -0.12 ± 0.11 °C).

The majority of thermoregulatory adaptations have been reported to occur within the first seven days of heat exposure (Guy *et al.*, 2015; Périard, Racinais and Sawka, 2015); however, in one study using an isothermic HA protocol, where thermal impulse was maintained, core temperature (oesophageal) was reported to be 0.20 °C lower after eight days and continued to lower to 0.32 °C after 22 days (Patterson, Stocks and Taylor, 2004). Suggesting that a LTHA can be advantageous for an individual to

undertake to induce further adaptations to reduce thermal strain, as resting core temperature continued to decline with longer exposure durations.

Resting skin temperature is unaffected by HA, but after HA mean exercising and end of exercise skin temperature has been reported to be lower than pre-acclimation values (Tyler *et al.*, 2016). Skin temperature has a similar time course for adaptation rate to core temperature, where this rate is rapid and occurs within seven days and skin temperature has not been found to lower with a longer exposure period (Patterson, Stocks and Taylor, 2004). The increase in skin blood flow in response to HA likely explains the mechanism that supports the lowering of skin temperature due to an increase in heat loss pathways, with heat transferred to the skin to be dissipated to the environment.

2.8.3.2. Cardiovascular responses to heat acclimation

After exposure to repeated heat stress, cardiovascular stability is improved and quantified by the reduction in HR responses during exercise in the heat (Patterson, Stocks and Taylor, 2004; Tyler *et al.*, 2016) from a combination of adaptations, which include an increase in plasma volume, enhanced sweating sensitivity and rate, skin blood flow and a better maintenance of fluid balance, all serving to benefit exercise performance in the heat (Lorenzo *et al.*, 2010).

Cardiovascular adaptations are typically reported to occur first and at a rapid rate, within 4 days to 5 days of exposure, continuing to complete

adaptation within seven days (Pandolf, 1998; Périard *et al.*, 2016; Tyler *et al.*, 2016) regardless of the ambient conditions HA is conducted in (Griefahn, 1997). When comparing MTHA to STHA protocols, no further adaptations have been observed on lowering resting HR, -5 ± 1 $b.min^{-1}$ and -5 ± 5 $b.min^{-1}$, respectively (Tyler *et al.*, 2016). Therefore, HA protocols lasting greater than seven days were not found to be more effective on reducing HR. However, to date there is limited evidence on the effectiveness of LTHA exposure on cardiovascular adaptations. In one LTHA study that used an isothermic model, Patterson, Stocks and Taylor (2004) reported resting HR to be lower after eight days (-5 $b.min^{-1}$) and this decline continued till the end of the HA period (22 days; -5 $b.min^{-1}$). This provides evidence that the magnitude of physiological adaptations to occur are greater with longer exposure periods in healthy individuals.

The increase in plasma volume (hypervolemia) elevates central circulatory blood volume (hypervolemia) and central venous pressure, allowing for a greater cardiac-filling and end-diastolic volume (Nose *et al.*, 1990; Convertino, 1991; Roy *et al.*, 2000), which is the mechanism responsible for lowering resting and during exercise HR. Hypervolemia occurs from an elevation in fluid-regulating hormones (aldosterone, arginine vasopressin (AVP) and atrial natriuretic factor) responsible for sodium and water retention. This response occurs in conjunction with increases in plasma transcription factor protein content (Fellmann, 1992), increasing the oncotic pressure within the vascular spaces to allow for a greater fluid retention. AVP governs free water retention while aldosterone causes renal

retention of water with sodium, in addition to regulating sodium of the sweat gland (Allsopp *et al.*, 1998; Stacey *et al.*, 2018). Hypervolemia occurs rapidly and can expand by a range of 3% to 27% (Périard *et al.*, 2016), depending on type of exercise, environmental conditions, intensity and duration of exercise, body posture, frequency of exercise and hydration status (Sawka *et al.*, 1983; Sawka *et al.*, 1984; Fellmann, 1992; Kenefick *et al.*, 2014). Plasma volume has been shown to be influenced by seasonal changes, with an expansion of 5% occurring in the hottest months and reduction of around 3% in the coldest months (Sawka and Coyle, 1999).

It is understood (and covered in section 2.4.1.3) that during exercise in the heat, blood flow is in competition between the muscle, skin and the maintenance of central blood volume and blood pressure, which causes an elevated HR and reduced stroke volume in order to maintain cardiac output. Another haematological adaptations mediated by an increased plasma volume is the increase in stroke volume, enhancing the ventricular filling while maintaining mean arterial pressure (Chalmers *et al.*, 2014). Nielsen *et al.* (1993) examined stroke volume responses before and after 9 days to 12 days of fixed-intensity (60% $\dot{V}O_{2max}$) HA in hot dry environmental conditions (40 °C, 10% rh). The authors reported that in conjunction with plasma volume expansion, stroke volume and cardiac output (~1.8 $L \cdot min^{-1}$) increased during exercise. In a follow up study, Nielsen *et al.* (1997) investigated 8 days to 13 days of the same fixed-intensity HA protocol but performed in a hot and humid environmental

condition (35 °C, 87% rh) on cardiac variables. In comparison to their earlier findings, stroke volume and cardiac output were not increased during exercise, even in the presence of plasma volume expansion. Potentially HA in a hot wet environment does not provide the same adaptive responses to some cardiac variables than during HA in a hot dry environment.

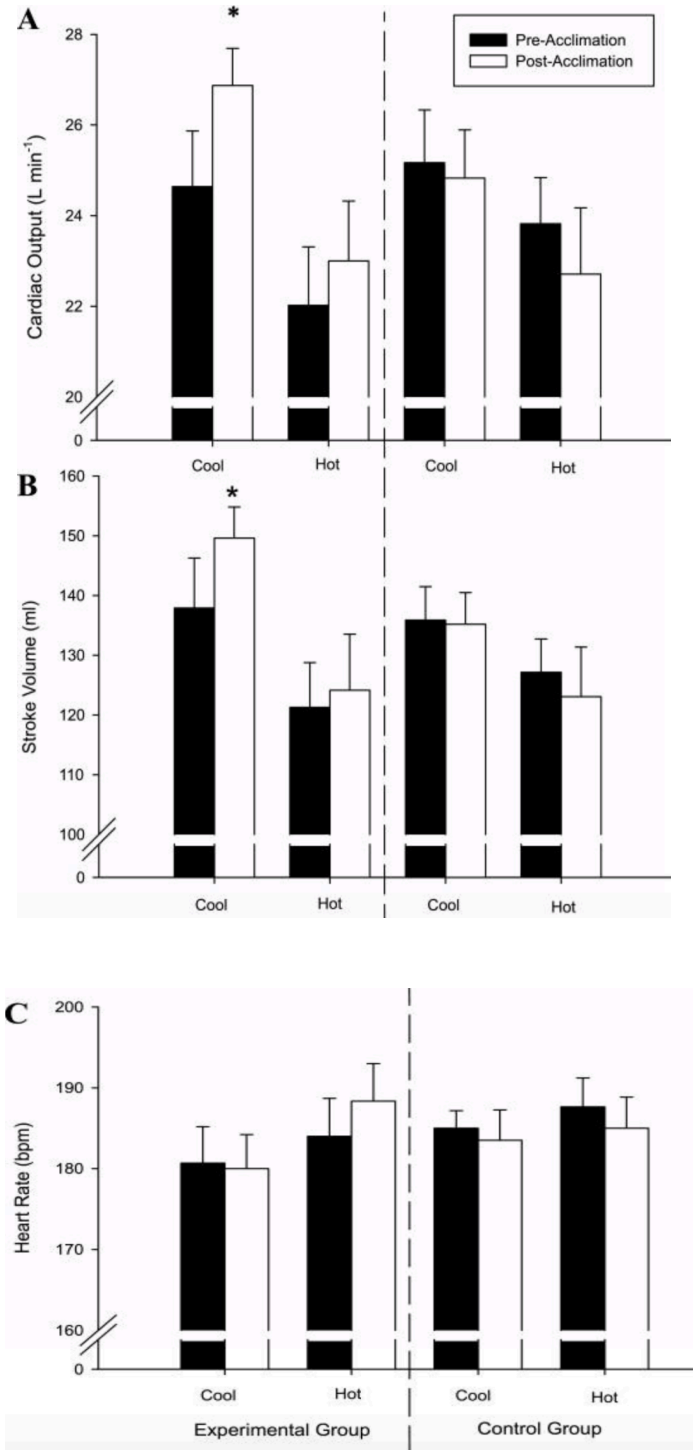

Figure 2.5. Heat acclimation effects on maximal cardiac output (A), and their corresponding stroke volume (B), and heart rate (C) during $\dot{V}O_{2max}$ test in a cool (13 ºC) and hot (38 ºC) environment. Values are mean ± SE for heat acclimation subjects and eight controls.

2.8.3.3. Sudomotor responses to heat acclimation

The importance of enhancing sudomotor function is primarily to enhance evaporative cooling to facilitate heat loss and reduce heat being stored. Unlike the rapid thermoregulatory and circulatory adaptations that occur from repeated heat exposure, STHA had either a small effect size (+5 ± 11%) (Tyler *et al.*, 2016) or no reported change in sudomotor function (Garrett *et al.*, 2009, 2014; Mee *et al.*, 2015; James *et al.*, 2017). After MTHA (+29 ± 29%) and LTHA (+33%), an increase in sweat rate has been observed, highlighting that there is a strong positive relationship between exposure duration and sudomotor adaptations (Tyler *et al.*, 2016). Adjustments to the onset of sweating have also been proposed, where sweating sensitivity is increased as individuals have an earlier onset of sweating to occur at a lower core temperature (Nadel *et al.*, 1974; Roberts *et al.*, 1977; Shvartz *et al.*, 1979). Nadel *et al.* (1974) observed that after ten consecutive days of HA participants core temperature threshold for sweating was reduced by -0.30 °C.

Along with the enhanced sweat rate and increased sweating sensitivity, sweat composition has also been observed to change with HA (Périard, Racinais and Sawka, 2015; Buono *et al.*, 2018). A number of different electrolytes are secreted in sweat, with sodium chloride highly influenced by sweat rate (Patterson, Galloway and Nimmo, 2000; Shamsuddin *et al.*, 2005; Buono, Ball and Kolkhorst, 2007). Although the exact physiological mechanism/s responsible for this remain uncertain, when sweat rate is low

approximately 85% of sodium is reabsorbed (Buono *et al.*, 2008). Furthermore, when increased above a certain sweat rate threshold, an incomplete sodium ion reabsorption from the distal duct can occur. The rate of reabsorption is dependent on skin temperature (Shamsuddin *et al.*, 2005), training (Amano *et al.*, 2017) and HA status (Kirby and Convertino, 1986; Buono, Ball and Kolkhorst, 2007).

Pre-acclimated individuals have been shown to have sodium concentrations of 60 $mEq \cdot L^{-1}$ or higher, where prolific sweating increases the amount of sodium lost in sweat. After a period of HA, sodium is reabsorbed within the duct of the sweat gland preserving sodium and reducing the amount lost in sweat (Sato and Dobson, 1970; Sato, Dobson and Mali, 1971). In a classic study, Kirby and Convertino (1986) reported a 50% reduction in sweat sodium concentrations after ten days of HA. In recent times, Buono *et al.* (2018) confirmed the findings of Kirby and Convertino (1986), as they observed a 45% reduction in sweat sodium ion concentration at any given sweat rate following seven days of HA. Buono *et al.* (2018) was one of the first to report the time course changes in sweat sodium concentrations and the authors reported that it was significantly reduced by day three of heat exposure. A more dilute sweat widens the water vapour gradient between skin and ambient air temperature making sweat more easily evaporated, allowing for more heat to be dissipated for temperature regulation (Périard, Racinais and Sawka, 2015; Baker, 2017; Buono *et al.*, 2018).

A possible mechanism responsible for the reduced sodium concentration in sweat after HA is the increase in circulating steroid hormone aldosterone. The effect of this increase is a greater expression and/or a translocation of the epithelial sodium channel into the apical membrane of the eccrine sweat gland duct (Kirby and Convertino, 1986; Booth, Johnson and Stockand, 2002; Quinton, 2007; Buono *et al.*, 2018). This potential mechanism has been supported through observing no change in sodium concentration in sweat in patients with Addison's disease, where the adrenal glands do not produce enough aldosterone (Robinson and Robinson, 1954). Furthermore, sudomotor function has also been shown to be different between sexes, with females having a reduced sweating ability during exercise in the heat (Gagnon and Kenny, 2011). It was once believed that females had a lower sweat sodium concentration compared to males; however, these studies did not take into consideration the sweat rate differences between sexes, which is lower in females (Ichinose-Kuwahara *et al.*, 2010). There are however, differences in trained females compared to trained males, whereby females have a lower sweat rate.

2.8.3.4. Metabolic responses to heat acclimation

Exercise performed in the heat is performed at a greater oxygen cost and accelerated lactate accumulation than during exercise in temperate environmental conditions (Fink, Costill and Van Handel, 1975; Dimri *et al.*, 1980). Dimri *et al.* (1980) associated the increase in oxygen demand to correspond to the increase in HR and pulmonary threshold, observed

with increasing thermal stress. Exercising in the heat before acclimation has been demonstrated to alter muscle metabolism and following a period of acclimation, there are some reversal changes (Febbraio *et al.*, 1994). In the available literature there has been a number of reported positive metabolic adaptations to occur as a result of HA. Further metabolic adaptations include glycogen sparing and Febbraio *et al.* (1994) observed pre-acclimated individuals to have a greater reliance on carbohydrate metabolism and a higher mean respiratory exchange ratio (RER). Alongside these changes, it was reported by Young *et al.* (1985) that individuals have an increase in muscle lactate accumulation when exercising in the heat at the same relative intensity, compared to when exercising in a cooler environment. However, a potential limitation to this work was that blood samples were obtained from venous blood to measure blood lactate, which reflects the balance between muscular production, efflux into the blood and removal from the blood and not necessarily the local muscular environment. With splanchnic blood flow reduced in the heat the removal of lactate may have been impaired rather than the production of lactate is increased as a result of exercise in the heat.

2.8.4. Perceptual responses to heat acclimation

The effect of increasing ambient air temperature on increasing RPE and thermal perceptions (Tucker *et al.*, 2006; Schlader, Stannard and Mündel, 2010; Borg *et al.*, 2018) were reviewed in section 2.4.2. The HA literature has predominately focused on the physiological adaptations to exercise in

the heat, and therefore, less is known on the effects of HA on behavioural thermoregulation. The relationship between RPE and HR response have been shown to be highly correlated (Scherr *et al.*, 2013). Therefore, it's not surprising that RPE has been shown to decrease after 3 days to 6 days of HA in conjunction with a reduction in cardiovascular and thermoregulatory strain (Pandolf, Burse and Goldman, 1977; Castle *et al.*, 2011; Tyler *et al.*, 2016). During a fixed intensity (50% $\dot{V}O_{2max}$) ten day HA protocol, Castle *et al.* (2011) observed that by the third day RPE was lower than compared to the first session in the heat. Furthermore, in a recent meta-analysis, Tyler *et al.* (2016) reported HA to have a moderate effect on reducing overall mean RPE during exercise; however, not all have reported a HA protocol to lower RPE (Lorenzo *et al.*, 2010).

When exercising at a fixed work rate, RPE increases over the duration of an exercise bout (Crewe, Tucker and Noakes, 2008) while on the other hand when exercise is set to a fixed RPE value, work output is reduced as exercise duration progresses (Tucker *et al.*, 2006). Crewe, Tucker and Noakes (2008) concluded that the rate of rise in RPE predicts the duration of exercise to exhaustion at a constant power output when exercising in different environments, which supports the importance of having a reduced RPE value following HA. In one investigation, Costa *et al.* (2014) had six male ultra-endurance runners complete a HA protocol consisting of six 2 h running bouts performed at 60% $\dot{V}O_{2max}$ in the heat (30 °C). On completion of the HA protocol, the ultra-endurance runners rated thermally more comfortable compared to pre-acclimation TC ratings.

Comfort during exercise is regulated by thermal perceptions and a comparison of sensations that the individual felt at rest (Bleichert *et al.*, 1973; Schlader, Stannard and Mündel, 2010). Gagge, Stolwijk and Hardy (1969) reported that sensations of temperature were principally related to skin and environmental temperature and less related to metabolic rate, muscle and core temperature. Thermal comfort has been defined as: "the condition of the mind which expresses satisfaction with the thermal environment" (Epstein and Moran, 2006). After a HA protocol, participants have reported to feel more comfortable and had a lower physiological strain (Sunderland, Morris and Nevill, 2008), which subsequently improved exercise performance in the heat. This continued reduction in TC from HA exposure, appears to play a key role in the selection of exercise intensity during performance tests.

2.8.5. Exercise performance after acclimation

In response to the numerous physiological and perceptual adaptations to occur from HA, individuals have a reduced overall strain compared to pre-acclimation measurements. As a result individuals are able to delay the effect of hyperthermia-induced decrements to exercise capacity and have a better maintenance of power output during a performance test (Lorenzo *et al.*, 2010; James *et al.*, 2017). Tyler *et al.* (2016) reported that the greatest improvements were observed during exercise capacity tests (which is not unsurprising due to the higher coefficient of variation (CV > 10%) (Currell and Jeukendrup, 2008) with an overall mean increase in

TTE by 23 ± 29%, compared to smaller performance outcomes of an overall mean increase in TT by 7 ± 7%. The greatest improvements identified to occur after completing a LTHA protocol (22 ± 29%) compared to when completing a STHA protocol (7 ± 8%). This finding was also supported by Guy *et al.* (2015) who conducted a meta-analysis comparing the performance data from STHA (n = 7) and MTHA (n = 7) protocols and reported 8 days to 14 days was more beneficial to performance and exercise capacity. With MTHA shown to increase TTE by 31 ± 29% and TT by 10.2 ± 14% compared to smaller improvements after STHA (TTE: 11 ± 8% and TT: 2.4 ± 3.5%).

During a TTE test in the heat (49 °C, 20% rh), Pandolf and Young (1992) reported 24 participants were unable to complete a 100 min walk on the first day of exposure. However, by day three 40% of participants successfully completed the walk, which continued to increase by day five, reaching an 80% completion rate, with all but one participant successful by day seven. Following a isothermic STHA protocol (five days), Garrett *et al.* (2009) reported a 14% improvement (106 s) on cycling TTE in a hot environment (32 °C, 60% rh). Similarly, James *et al.* (2017) reported that 5 km TT was improved by 6.5% and concluded that a cycling isothermic STHA protocol was effective for improving running performance in hot and humid conditions (32 °C, 60% rh). Compared to STHA, greater improvements in exercise capacity have been observed after a MTHA period, for example Nielsen *et al.* (1993) reported after 9 days to 12 days of HA in a hot dry environment (40 ºC to 42 °C, 10% to 15% rh), TTE

doubled (from 48 ± 1.9 min to 80 ± 3.3 min). During performance tests, early work by Sawka *et al.* (1985) reported that maximal power increased by 2% (267 vs. 272 W) after nine days of HA in a hot dry environment (49 °C, 20% rh). More recently, Lorenzo *et al.* (2010) investigated the effect of a ten day HA protocol on 1 h cycling TT performance in the heat (38 °C, 30% rh), and reported a 7.4% improvement in TT performance (776.2 ± 50.9 kJ vs. 718.7 ± 42.3 kJ), where participants completed a greater amount of work (kJ) from pre-acclimation baseline testing (Figure 2.8).

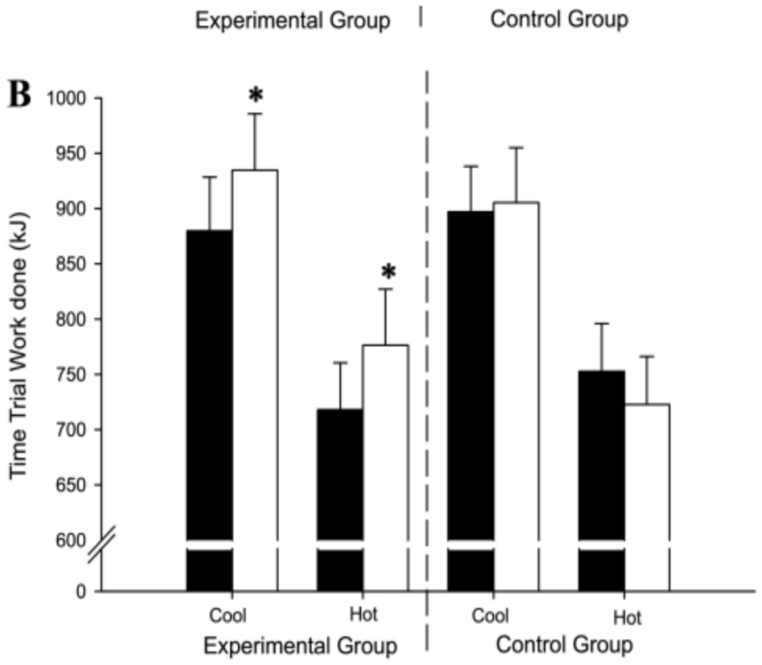

Figure 2.6. The effect of heat acclimation on total work done (kJ) during a 1 h time trial (B). Values are means ± SE for twelve heat acclimation subjects and eight controls *P < 0.005 vs. pre-acclimation within environmental condition. [Reproduced from Lorenzo *et al.* (2010), with permission].

It seems reasonable to suggest that the performance benefits are not always progressive with greater heat exposure in highly trained individuals and during a TT performed outside of the laboratory (Figure 2.9). In one study, Racinais *et al.* (2015) reported that after six days of heat exposure, 43.4 km TT in the heat (TTH) was improved but no overall further differences were observed in TT performance after 14 days, in highly trained cyclists.

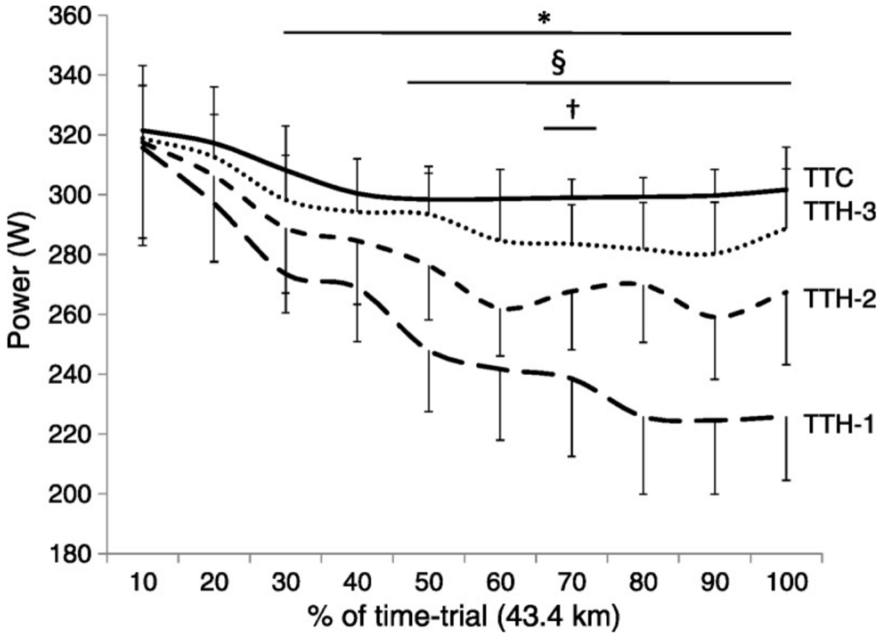

Figure 2.7. Power output (W) during a 43.4 km cycling TT in TTC (plain line) and in TTH-1 (long dashed line), TTH-2 after 5 days (short dashed line), and TTH-3 after 13 days (dotted line). Data are mean ± SD. *§†TTC was significantly ($P < 0.05$) higher than TTH-1, TTH-2, and TTH-3, respectively. [Reproduced from Racinais *et al.* (2015), with permission].

2.8.6. Cognitive function in response to heat acclimation

It is well established that HA induces a number of physiological and behavioural adaptations, as discussed in detail above (section 2.8). However, there are few studies that have examined the effect of HA on cognitive function in hot environmental temperatures (Gaoua, 2010) and

the lack of systematic approach makes it challenging to conclude whether repeated heat exposure has a positive protective effect on cognitive function. In one study, Radakovic *et al.* (2007) investigated the effects of ten days of passive or active HA (3 h of heat exposure each day in 35 °C) on cognitive function, using the computerised cognitive function test (Cambridge Neuropsychological Test Automated Batteries attention battery). The authors reported that regardless of acclimation status (passive and active), simple task performance was unaffected but ten days of HA prevented the detrimental effects of heat stress on more complex attentional performance. Radakovic *et al.* (2007) also observed no differences between passive or active HA when reporting improvements in cognitive function, however the data presented makes it challenging to determine whether physiological adaptations were induced from HA. In more recent work, Racinais *et al.* (2017) reported that 11 days of passive HA (participants were exposed to 48 ºC to 50 ºC, 50% rh for 60 min a day) reduced thermal strain and protected attention tasks by decreasing impulsivity (i.e. reaction time) and allowing for a better accuracy. **In Chapter 6 of this thesis, cognitive function was investigated pre- and post- 45 min heat stress test (40 °C, 50% rh) and after a 60 min isothermic HA protocol (target rectal temperature ~38.5 °C) over performed daily over a short-term (five days) and medium-term (ten days) time course.**

2.8.7. Gastrointestinal response to heat acclimation

Limited evidence exists on interventions to prevent increased GI permeability reported during exercise in the heat (see section 2.4.1.4). To date, the literature has focused upon the use of nutrition and supplementation interventions, yet this remains poorly understood and warrants further research (Guy and Vincent, 2018). In recent years, limited research has investigated the effects of STHA as a protective intervention to reduce the exercise-induced endotoxin leakage (Kuennen *et al.*, 2011; Barberio *et al.*, 2015; Guy *et al.*, 2016). The literature thus far investigating the effect of HA on exertional-endotoxemia is inconclusive (Kuennen *et al.*, 2011; Barberio *et al.*, 2015; Guy *et al.*, 2016), with methodological issues and inconsistencies arising from the studies referenced. LPS responses appear to be associated with the type, intensity and duration of exercise, because while neither Kuennen *et al.* (2011) nor Guy *et al.* (2016) observed any changes in circulating LPS concentrations during and after a low intensity cycling exercise protocol for ~40 min to 45 min (~55% $\dot{V}O_{2max}$), Barberio *et al.* (2015) did report within session elevations following higher intensity (~78% $\dot{V}O_{2max}$) running in the heat until exhaustion or a set increase in core temperature (+2 °C). These data suggest that one factor required to observe a measurable LPS response is to exceed a physiological strain above a certain threshold. However, this threshold is currently unknown and none of these studies observed a beneficial effect of STHA (<7 days) on LPS response. In the studies by Kuennen *et al.* (2011) and Guy *et al.* (2016), this is unsurprising because

they did not observe a within session increase in LPS. Controversially, Barberio *et al.* (2015) reported that this response was not attenuated by HA, possibly because the STHA protocol investigated provided a cumulative physiological strain below the theorised threshold. Barberio *et al.* (2015) investigated a five day HA protocol using a set increase in core temperature (+2 °C). A potential limitation to using this is that as adaptation occurs and resting core temperature lowers, individuals may not be reaching a sufficient thermal strain relative to the start of HA and/or the HA regimen may not have been long enough to induce adaptation. Therefore, it is yet to be concluded whether there is a cumulative strain rather than an acute physiological strain that is linked to the translocation of LPS into the systemic circulation during exercise in the heat, resulting in the development of exertional heat illness. **Therefore, the aim of Chapter 7 was to investigate whether an acute bout of exercise under thermal strain would induce an endotoxin LPS response in moderately trained individuals to exercising in the heat. Secondly, whether the endotoxin LPS response to occur from the first heat exposure session would decline after a short-term (five days) daily 60 min isothermic (target rectal temperature of ~38.5 °C) HA protocol and whether medium-term (ten days) HA was more effective than short-term HA.**

2.8.8. Summary on heat acclimation

In summary, HA reduces the thermal strain at a given exercise intensity during exercise performed in hot environmental conditions. So far a number of HA protocols have been investigated; however, it is yet to be concluded what the most effective and efficient protocol for an athlete to undertake prior to competition in the heat is. For physiological and perceptual adaptations to occur, a heat stress must induce a physiological strain above an adaptation threshold, proposed to be the attainment and maintenance of a rectal temperature of ~38.5 ºC. Recent evidence has supported that a 90 min daily isothermic HA protocol over five and ten days is a successful method at eliciting the thermal adaptations, however, yet to be investigated is whether this protocol would induce these adaptations when exposed to 33.3% less time per day (60 min). To date, there is inconclusive literature that exists on the effects of heat stress on cognitive function, with some evidence shown that the increase in thermoregulatory strain has detrimental effects on cognition and fewer investigations have explored the influence of HA providing protection to the decline on cognitive function during heat stress. Far less is known on the response that an isothermic HA protocol has on the LPS response, whether the increase in physiological strain at the onset of HA protocol would induce an increase and sustain rise in circulating LPS response during HA. **The aim of experimental study 3 presented in Chapters 5 to 7 of this thesis was to investigate whether a daily 60 min isothermic HA protocol would reduce the physiological and perceptual strain**

experienced when exercising in the heat. It also sought to investigate whether there was a time course effect on these responses over a five and ten day period, without inducing an endotoxin LPS response. An additional aim was to investigate whether a daily isothermic HA protocol (target rectal temperature ~38.5 ºC) would offer protection to the impairments in cognitive function when under thermal stress.

2.9. Aims and hypotheses

The aim of this PhD thesis was to investigate the effects of perception and expectation of hydration status on exercise capacity and performance in the heat; pre- and per-cooling in isolation and in combination on exercise capacity and performance in the heat; and the effects of a daily 60 min isothermic HA protocol on the physiological, perceptual, endotoxin and cognitive function response.

Chapter 3 - The Influence of Perception and Expectation of Hydration Status on Cycling Performance in the Heat.

Research aim:

1. To investigate the effects of perceived hydration status on exercise performance under heat stress, when blinded to the true nature of the study protocol via the use of a nasogastric tube.

Hypotheses:

H_0: The amount of work completed during 15 min cycling time trial (TT) will not differ between trials when participants are informed that they are hypohydrated compared to when they are told that they are euhydrated.

H_1: The amount of work completed during 15 min cycling time trial (TT) will be impaired when participants are informed that they are hypohydrated compared to when they are told that they are euhydrated.

Chapter 4 – The Effects of Pre- and Per-Cooling Interventions on Cycling Performance in the Heat in Highly Trained Cyclists and Triathletes.

Research aim:

1. To investigate the effect of isolated and combined pre- and per-external cooling methods on cycling performance in the heat in well-trained athletes.

Hypotheses:

H_0: neither isolated nor combined pre- and per-cooling interventions would increase distance covered during 15 min time trial (TT) cycling performance.

H_1: isolated and combined pre- and per-cooling interventions would increase distance cycled during 15 min TT by sustaining a higher self-selected power output during the TT with performance improvements seen during the latter

stages of the TT (pre-cooling by reducing physiological and perceptual strain and per-cooling by reducing the perceived thermal strain).

H_2: combined pre- and per-cooling would be the most effective approach as a result of a cumulative and longer-lasting benefit.

Chapter 5 – Short-term Isothermic Heat Acclimation Elicits Beneficial Adaptations but Medium-term Elicits a more Complete Adaptation.

Research aim:

1. To investigate whether a 60 min daily isothermic (target rectal temperature of ~38.5 °C) HA regimen would reduce the physiological and perceptual strain experienced when exercising in the heat.
2. To investigate if there was a time-course effect on the physiological and perceptual adaptations and whether MTHA was more effective than STHA.

Hypotheses:

H_0: the isothermic HA protocol would not provide a sufficient thermal impulse to induce positive changes in physiological and perceptual measurements.

H_1: the isothermic HA protocol would provide a sufficient thermal impulse to induce positive changes in physiological and perceptual measurements.

H_2: adaptations to the physiological and perceptual systems would be more complete following MTHA than STHA.

Chapter 6 – The effects of a Short-term and Medium-term Isothermic Heat Acclimation Protocol on Cognitive Function.

Research aim:

1. To investigate whether an acute bout of exercise is detrimental to cognitive function when measured from pre- to post-exercise under heat stress
2. To investigate whether a daily 60 min isothermic (target rectal temperature of ~38.5 °C) HA protocol would offset the detrimental effects of heat stress during exercise on cognitive function in moderately-trained endurance individuals.
3. To determine if there was a time-course effect on cognitive function, and whether MTHA was more effective than STHA.

Hypotheses:

H_{01}: an acute bout of exercise under heat stress would not impair cognitive function when measured from pre- to post-exercise.

H_1: an acute bout of exercise under heat stress would impair cognitive function when measured from pre- to post-exercise.

H_{02}: an isothermic HA protocol has no effect on offsetting the detrimental effects of exercise heat stress on cognitive function.

H_2: an isothermic HA protocol would offset the detrimental effects of exercise heat stress on cognitive function.

H_3: this outcome would be greater following MTHA than STHA.

Chapter 7 – The Effects of a Short-term and Medium-term Isothermic Heat acclimation Protocol on the Lipopolysaccharide (LPS) Response to Exercise under Heat Stress.

Research aim:

1. To investigate whether an acute bout of exercise under high thermal strain would induce the endotoxin LPS response to when exercising in the heat in moderately-trained endurance individuals.
2. To determine if the endotoxin LPS response to occur from the first heat exposure session would decline after a daily STHA isothermic protocol (target rectal temperature ~38.5 ºC)
3. To determine if there was a time-course effect on LPS response, and whether MTHA was more effective than STHA.

Hypotheses:

H_{01}: an acute bout of exercise under heat stress has no effect on LPS concentrations pre- to post-HST.

H_1: an acute bout of exercise under heat stress increases LPS concentrations when measured from pre- to post-HST.

H_{02}: a daily 60 min isothermic HA protocol would have no effect on circulating endotoxin LPS concentrations.

H_2: a daily 60 min isothermic HA protocol would reduce circulating endotoxin LPS concentrations.

H_3: this response would be greater following MTHA than STHA, where MTHA would potentially offer more complete adaptations to the cardiovascular and thermoregulatory systems.

Data collected from the first experimental study are presented in Chapter three. Data collected from the second experimental study are presented in Chapter four. Data from the third experimental study are split into three Chapters presented in Chapters five, six and seven. The experimental chapters follow the time-line of when these experimental studies were conducted, with Chapter three investigated at Loughborough University under the supervision of Dr Lewis James before Chapters four to seven were conducted at the University of Roehampton under the supervision of Drs Richard Mackenzie, Steven Trangmar and Chris Tyler.

Chapter 3. **The Influence of Perception and Expectation of Hydration Status on Cycling Performance in the Heat**

3.1. Introduction

Exposure to environmental and metabolic heat stress elevates skin and core temperatures, with concomitant increases in sweat rate and cutaneous vasodilation to increase heat dissipation (Akerman *et al.*, 2016; Cramer and Jay, 2016). When exercise is performed in uncompensable heat stress conditions, sweat rate often exceeds fluid consumption, resulting in a body water deficit referred to as hypohydration within this thesis (Sawka, 1992). Exercise-induced hypohydration results in a reduction in plasma volume (hypovolemia) and an increase in plasma osmolarity (hyperosmolarity) and both can contribute to an increase in cardiovascular and thermal strain, due to the impaired ability to dissipate heat (Cheuvront *et al.*, 2010; Berkulo *et al.*, 2016). As discussed in detail in the literature review (see section 2.6.5 & 2.6.6), hypohydration results in a greater physiological and perceptual strain, which often leads to a decrease in pacing consistency (Stearns *et al.*, 2009) and therefore, an overall impairment in exercise performed in the heat (Cheuvront *et al.*, 2005; Kenefick *et al.*, 2010; Logan-Sprenger *et al.*, 2015) compared to when exercising in an euhydrated state (Cheuvront *et al.*, 2005).

Despite previous evidence highlighting the detrimental effects of exercising with hypohydration in the heat, the methodology used to induce hypohydration has raised a number of concerns on the potential influence this may have had on performance outcomes (Cotter *et al.*, 2014; James *et al.*, 2017). Firstly, from a physiology standpoint, different methodologies

used to induce hypohydration have altered the redistribution of water between the intracellular and the extracellular compartments. With diuretic-induced hypohydration resulting in iso-osmotic hypervolemia, where a greater amount of fluid is lost from the extracellular compartments, which is different from water deficits that occur from sweating. As sweat is typically hypotonic relative to plasma, plasma becomes hyperosmotic when sweat output accounts for hypohydration (Sawka, Cheuvront and Kenefick, 2015). Secondly, the overtness of the methods used to induce hypohydration (e.g. fluid restriction) has meant that participants are likely aware and not blinded to their experimentally manipulated state (e.g. euhydrated or hypohydrated). The lack of blinding potentially contributes to a nocebo effect on performance outcomes when investigating hypohydration, as participants may have an expectation that hypohydration impairs exercise performance (McClung and Collins, 2007). As well as an awareness of the true experimental protocol, the restriction of fluid often results in an increase in thirst sensation, due to an increased secretion of vasopressin in response to hyperosmotic hypovolemia (Sawka *et al.*, 1985). It has been proposed that the sensation of thirst is one of the driving factors modulating exercise intensity, acting as part of the anticipatory regulatory system (Sawka and Noakes, 2007; Noakes, 2010); however, with the development of a new methodology, where thirst is controlled but hypohydration still remains, more recent data have shown that exercise performance is impaired in the absence of thirst (Adams *et al.*, 2018).

The expectation and perception of receiving a certain treatment/intervention may in itself have an effect on performance (Clark *et al.*, 2000; Beedie *et al.*, 2006). The belief of treatment could override peripheral and central fatigue to the extent to which exercise performance is improved (Piedimonte, Benedetti and Carlino, 2015). Different methods have been used to blind participants to their hydration status and, in the same year, both Cheung *et al.* (2015) and Wall *et al.* (2015) manipulated hydration status through intravenous saline solutions. While this approach successfully blinds participants to hypohydration, replacing sweat losses with the infusion of isotonic saline, results in plasma osmolarity remaining elevated (i.e. hyperosmotic) versus when hypohydrated (i.e. hypertonic hypovolemia), regardless of the actual hydration status. This issue can be overcome by using a nasogastric tube to blind participants of their true hydration status. Results from two recent nasogastric tube studies, in which participants were unaware that their hydration status was being manipulated (James *et al.*, 2017; Funnell *et al.*, 2019), demonstrated that hypohydration impairs exercise performance as a result of the physiological consequences of ~2% to 3% hypohydration rather than awareness of methods used to induce hypohydration. It is interesting to note that the findings from both exit interviews suggest that the blinding method used was successful, as participants were not fully aware of the nature of the hydration deception (James *et al.*, 2017; Funnell *et al.*, 2019). Whilst data are limited, it seems that the use of a nasogastric tube is successful in blinding participants from their true hydration status and allowing for hydration status to be manipulated, while eliciting the

physiological and perceptual consequences of exercise-induced hypohydration.

While the effects of manipulating hydration status using a nasogastric tube has been investigated, no study has investigated the effect of manipulating perceived hydration status on pacing strategy and cycling performance in the heat in hypohydrated participants despite data showing the role that expectations and prior knowledge can have. Therefore, the aim of the present study was to investigate the effects of perceived hydration status on cycling performance under heat stress, when blinded to the true nature of the study protocol via the use of a nasogastric tube.

It was hypothesised that:

H_0: The amount of work completed during 15 min cycling time trial (TT) will not differ between trials when participants are informed that they are hypohydrated compared to when they are told that they are euhydrated.

H_1: The amount of work completed during 15 min cycling time trial (TT) will be impaired when participants are informed that they are hypohydrated compared to when they are told that they are euhydrated.

3.2. Methods

3.2.1. Participants

Seven, recreationally active, non-heat acclimated males (mean ± SD: age 24 ± 2 y, stature 176.5 ± 3.8 cm, body mass 78.0 ± 10.4 kg and peak oxygen uptake ($\dot{V}O_{2peak}$) 53.0 ± 4.1 $mL \cdot kg^{-1} \cdot min^{-1}$) participated. Before participation, all participants completed a health screening questionnaire and received written details outlining the nature of the study and any risks and discomforts associated with taking part, before giving their written, fully-informed consent to participate. To blind the participants from the true nature of the study, participants were told that the study was investigating drinks of different compositions of carbohydrate and protein, and that the nasogastric tube was used as they would have been able to identify the drinks based on their flavour. This study was approved by Loughborough University's ethics committee (R15-P045, Appendix A3) and all procedures and protocols adhered to the guidelines of the World Medical Association (*Declaration of Helsinki*). For calibration measurements of equipment used see Appendix E1

3.2.2. Experimental design

Participants visited the laboratory on five occasions for three preliminary trials and two experimental trials. Each experimental trial required participants to cycle intermittently for 120 min at 50% peak power output

(PPO) (8 blocks of 15 min; 10 min cycling with 5 min of seated rest) before completing a 15 min TT in one of two conditions:

1. Targeted 2% hypohydration but told euhydrated (HYP-NT).

2. Targeted 2% hypohydration and told (HYP-T).

All trials were performed in the heat (35 °C, 60% relative humidity (rh); 2 $m.s^{-1}$ wind speed) at the same time of day (standardised within participant and between 08:00 – 09:30) in a randomised and counterbalanced order, separated by ~7 days. Environmental conditions were chosen based upon piloting whereby sweating was induced and fluid was infused to result in a body water deficit of ~2%, without having to infuse a high volume of water to replace sweat rates, this was important to avoid any gastrointestinal distress. Targeted 2% water deficit was based upon the statement from the American College of Sports Medicine (ACSM) position stand for exercise and fluid replacement that "Hypohydration >2% impairs exercise performance" (Sawka *et al.,* 2007). A 15 min TT has been previously demonstrated from published and unpublished data from our lab to demonstrate a high level of reliability with a preloaded trial and without a preloaded trial.

3.2.3. Preliminary testing

Stature (Harpenden Stadiometer, Holtain Ltd, UK) and body mass (AFW-120K, Adam Equipment Co., UK) were recorded before $\dot{V}O_{2peak}$ and PPO

was determined in ambient laboratory conditions using an incremental exercise test (starting at 95 watts (W) and increasing by 35 W every 3 min) until volitional exhaustion, on an electromagnetically braked cycle ergometer (Lode Corival, Groningen, Holland). Expired air samples (using Douglas bags), heart rate (HR) (Polar Electro Ltd., Kempele, Finland), and rating of perceived exertion (RPE; Borg, 1982) were measured and rated at the end of each stage, and at exhaustion. On completion of the exercise test, participants were familiarised with the insertion of the 8 g nasogastric tube (Sonde Gastro-Duodenal Typer Levin, Vygon Ltd., UK) which was first demonstrated by the investigator. On the second visit, participants were familiarised with two out of the eight stages of preload protocol (10 min of exercise (50% PPO) and 5 min seated rest) in the controlled environment followed by the 15 min TT performance test (90% PPO). Body mass (BM) was measured pre- and post- each stage and pre- and post- the TT to calculate sweat losses (sweat losses = pre-trial BM – post-trial BM), to determine the water (H_2O) volume provided during the experimental trials. On the third preliminary visit, participants were familiarised with the full experimental protocol (see 3.2.5), with the inclusion of the nasogastric tube.

3.2.4. Pre-trial standardisation

Prior to each experimental trial, participants were provided with lunch, dinner and snacks (8 g·kg BM^{-1} carbohydrate; 40 mL H_2O·kg BM^{-1}) and a pre-trial breakfast was consumed 1.5 h before arrival at the laboratory.

The prescribed breakfast provided 7 mL·kg BM^{-1} of fluid and 1 g·kg BM^{-1} of carbohydrate. These standardised fuelling strategies were based upon the necessary amount of carbohydrate needed to replenish glycogen stores and ensure participants began the experimental trials in a similar carbohydrate availability state (Burke *et al.*, 2011). Participants refrained from strenuous activity and alcohol and caffeine intake, 48 and 24 h prior to each trial, respectively. A telemetry pill (CorTemp® Ingestible Core Body Temperature Sensor) was ingested the night before at ~10 pm for measurement of gastrointestinal core temperature (T_{GI}) during experimental trials.

3.2.5. Experimental trials (Figure 3.1)

Upon arrival, a urine sample was collected to immediately determine osmolality (Osmocheck Digital Refractometer, Vitech Scientific Ltd, Partidge Green, UK) with a urine osmolality of < 900 mOsm·kg H_2O^{-1} required for trial participation (Armstrong *et al.*, 2010), before participants self-recorded nude body mass. No participant produced a urine sample of > 700 mOsm·kg H_2O^{-1}. Thereafter, a 20-gauge cannula was inserted into an antecubital vein (Becton Dickinson Venflon, Helsingborg, Sweden), before participants orally self-inserted the nasogastric tube to the depth estimated to reach the base of their stomach. The nasogastric tube was placed behind the ear and taped (Transpore, 3M Health Care, St Paul, MN) onto the upper back out of sight from the participant. After baseline procedures were complete, the participant was verbally informed with

standardised instructions about which experimental trial they were about to participate in (either “You will be on the euhydrated trial” or “You are on the 2% dehydration trial”). Measurements of 1) thirst, 2) stomach bloating and 3) fullness were each rated at baseline before the preload started, post-preload and post-TT using a 100-mm visual analogue scale: “How full do you feel now?,” with anchors of “not at all” and “extremely” placed at 0 mm and 100 mm, respectively. Participants then entered the controlled environment (35 °C and 60% rh) (Eurotherm 2704, Weiss-Gallenkaup, UK) and after 15 min of seated rest on the cycle ergometer, a pre-exercise blood sample was drawn (10 mL), HR and T_{GI} were recorded.

Participants then completed 120 min of intermittent exercise, which comprised of 8 blocks of 15 min; each block consisted of 10 min cycling exercise at 50% PPO (144 ± 15 W) with 5 min of seated rest off the cycle ergometer. Throughout the preload, to prevent unintentional internal cooling, warm water (total of 393 ± 236 mL, 35.4 ± 0.2 °C) was infused with a syringe into the stomach through the gastric feeding tube by the researcher to reach mild hypohydration of 1.5 - 2% BM. During the last 30 s of exercise in each exercise block, HR, T_{GI}, RPE, and thermal comfort (TC; Gagge, Stolwijk and Hardy, 1967) were recorded. Participants received additional water to orally ingest ~0.3 mL·kg BM^{-1} (total of 247 ± 32 mL, 19.0 ± 1.1 °C) at the end of each 10 min exercise block. On completion of the preload exercise, the nasogastric tube was removed, and nude BM was measured behind a protected screen in the controlled environment. Participants were then informed again of their actual or

deceived hypohydration status using the appropriate standardised instruction (either "You are euhydrated" or "You are dehydrated by 2%") once BM had been recorded and instructions were repeated before the TT commenced.

3.2.6. Time trial performance test (TT)

Participants were given standardised verbal instructions to complete as much work (kJ) as they possibly could within 15 min. Power output was initially set at 90% PPO (261 ± 31 W), and participants could increase or decrease the workload as desired by pressing the up or down button on the ergometer's console. No motivation was given to the participants and the only feedback provided was the time remaining. A screen separated the participant from the researcher to minimise distractions, every 5 min work completed (kJ), HR and T_{GI} were recorded without disturbing the participant. On completion of the TT, a final blood sample was

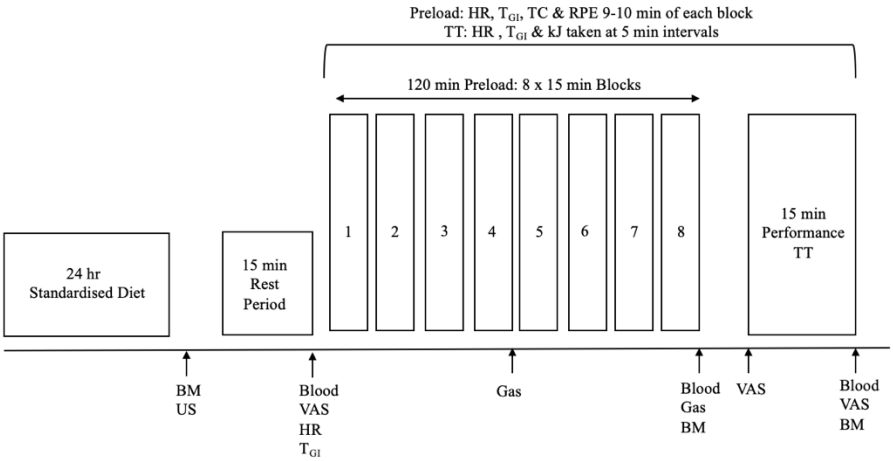

Figure 3.1. Experimental design. (HR: heart rate; T_{GI}: gastrointestinal core temperature; BM: body mass; kJ: work output; RPE: rating of perceived exertion; TC: thermal comfort; Gas: 1 min expired air samples; VAS – thirst, bloating and fullness ratings).

immediately drawn before the participant exited the controlled environment and towel dried before final nude BM was recorded.

3.2.7. Blood and gas analysis

For each blood sample, 5 mL of blood was dispensed into a tube containing a clotting catalyst (Sarstedt AG & Co., Nümbrecht, Germany) and 2.5 mL was mixed with EDTA (1.75 $mg \cdot mL^{-1}$; Sarstedt AG & Co., Nümbrecht, Germany), before plasma were then separated by centrifugation (1700 g at 4 °C for 10 min). The remaining 2.5 mL of blood was mixed with EDTA and then used for the determination of haemoglobin concentration (cyanmethemoglobin method) and haematocrit (microcentrifugation). Haemoglobin and haematocrit values were used to estimate changes in plasma volume and blood volume relative to the pre-exercise sample (Dill and Costill, 1974). Expired air samples (1 min) were collected at the end of the fourth and eighth exercise block during the intermittent exercise bout using the Douglas bag method and subsequently analysed (1400 series, Servomex, East Sussex, UK; Harvard Dry Gas Meter, Harvard Ltd., Kent, UK).

3.2.8. Statistical analysis

Data were analysed using SPSS (version 26, SPSS Inc.). Parametric assumptions were met unless stated otherwise. A one-way ANOVA was conducted to evaluate differences between trials for the distance cycled, hydration status and blood samples, and two-way repeated measures

ANOVA were performed to evaluate differences between trials for pacing, thermoregulatory, cardiovascular, expired air and perceptual variables. Where the assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimate. Where significant outcomes were present, post-hoc tests with Bonferroni corrections were performed. The alpha level was $P < 0.05$. Data are presented as mean ± SD.

3.3. Results

3.3.1. Hydration status – Table 3.1

There were no differences between trials for pre-trial BM ($P = 0.508$) or urine osmolality ($P = 0.117$). Pre-trial urine osmolality was < 700 $mOsm.kg^{-1}$ in both trials. After the preload, total BM losses were similar between both trials ($P = 0.265$), with participants starting ($P = 0.358$) and finishing ($P = 0.484$) the TT at a similar magnitude of hypohydration in both experimental trials. The overall mean total percentage of BM loss was not different between trials ($P = 0.454$). All data are presented below in Table 3.1. Changes in plasma volume (PV) ($P = 0.176$) and blood volume (BV) ($P = 0.318$) were similar between HYP-NT and HYP-T.

Table 3.1. Hydration status during hypohydration not told (HYP-NT) and during hypohydration told (HYP-T) experimental trials.

	HYP-NT	HYP-T
Pre-exercise BM (kg)	77.53 ± 9.47	77.74 ± 10.12
Pre-TT BM loss (%)	-1.66 ± 0.11	-1.64 ± 0.21
Post-TT BM loss (%)	-0.77 ± 0.12	-0.73 ± 0.13
Total BM loss (%)	-2.45 ± 0.15	-2.53 ± 0.30
Change in PV (%)	3.65 ± 12.36	-4.21 ± 4.75

Mean ± SD data for participants (n = 7).

3.3.2. Time trial - Physiological responses and performance data

There were no differences between trials in the total amount of work completed during the TT during HYP-NT and HYP-T (P = 0.130). The total mean work completed was 184.2 ± 35.5 kJ and 175.0 ± 36.7 kJ during HYP-NT and HYP-T, respectively (**Error! Reference source not found.**). Participants completed 9.2 ± 13.9 kJ (5.5 ± 6.9%) more work during HYP-NT but the difference was not statistically significant. Five (out of the seven) participants performed less work (-13.8 ± 13.7 kJ) and two performed more work (2.5 ± 1.2 kJ) during HYP-T. When the performance test was separated int o 5 min blocks (0 to 5 min, 5 to 10 min and 10 to 15 min), no differences were observed in the amount of work completed between trials at any 5 min time block (P = 0.130). There were main effects of time for HR (P < 0.001) (Figure 3.3) and T_{GI} (P = 0.031) (Figure 3.4) with all increasing progressively throughout the TT. During

the TT there were no main trial (P = 0.968, P = 0.673) or interaction (P = 0.381, P = 0.459) effects for HR or T_{GI}.

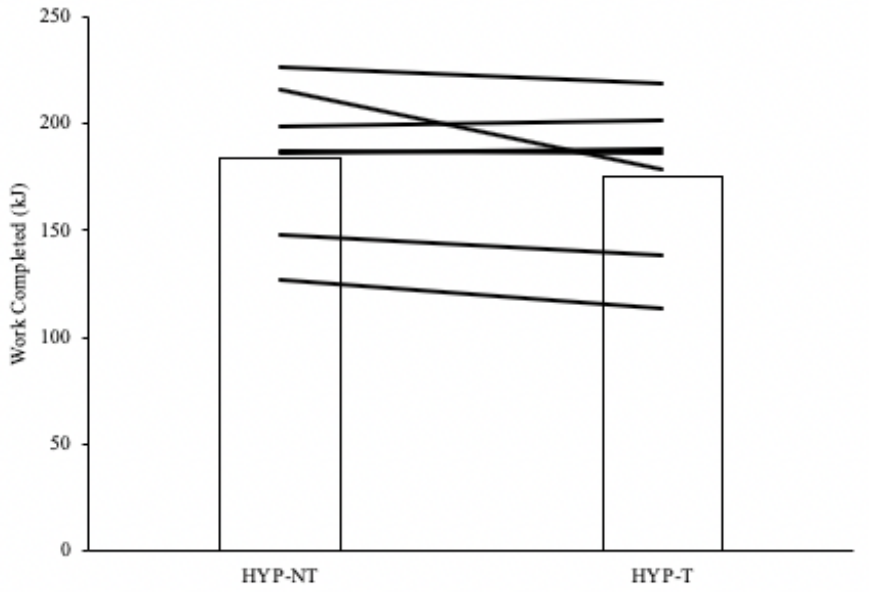

Figure 3.2. Mean work completed (kJ) during the 15 min TT performance test during not told (HYP-NT) and hypohydration told (HYP-T). Lines represent individual performance points during HYP-NT and HYP-T (n = 7).

3.3.3. Preload- Physiological Responses

There were main effects of time for HR and T_{GI} (all $P < 0.001$), all increasing progressively throughout the preload. During the preload there were no main effect of trial ($P = 0.171$, $P = 0.350$) nor was there an interaction effect ($P = 0.333$, $P = 0.680$) for HR or T_{GI}.

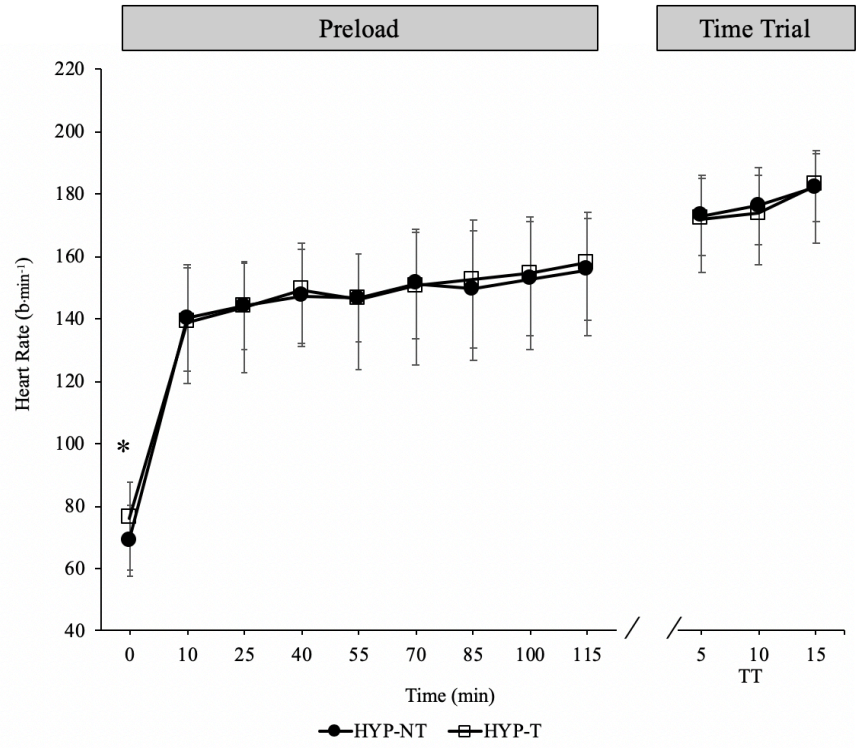

Figure 3.3. Heart rate (HR) response during the 120 min intermittent preload cycling exercise and during the 15 min time trial (TT) performance test in HYP-NT and HYP-T. Data are mean ± SD (n = 7). *Significant ($P < 0.05$) difference between trials.

Baseline measurements of HR were higher in HYP-T compared to HYP-NT ($P = 0.048$) (Figure 3.3), while T_{GI} was similar in both trials ($P = 0.749$) (Figure 3.4). At the end of the preload, participants had a similar level of cardiovascular (HYP-NT: 152 ± 17 $b.min^{-1}$; HYP-T: 155 ± 13 $b.min^{-1}$, $P = 0.167$) and thermoregulatory (HYP-NT: 38.02 ± 0.41 °C; HYP-T: 37.94

± 0.43 °C, P = 0.629) strain in both trials. There were no main effects of trial for $\dot{V}O_2$ (trial: P = 0.061; time: P = 0.458; trial*time interaction: P = 0.110). Respiratory exchange ratio (RER) was also similar between trials (P = 0.337), with no trial by time interaction effect (P = 0.646) but RER decreased during trials (P = 0.023).

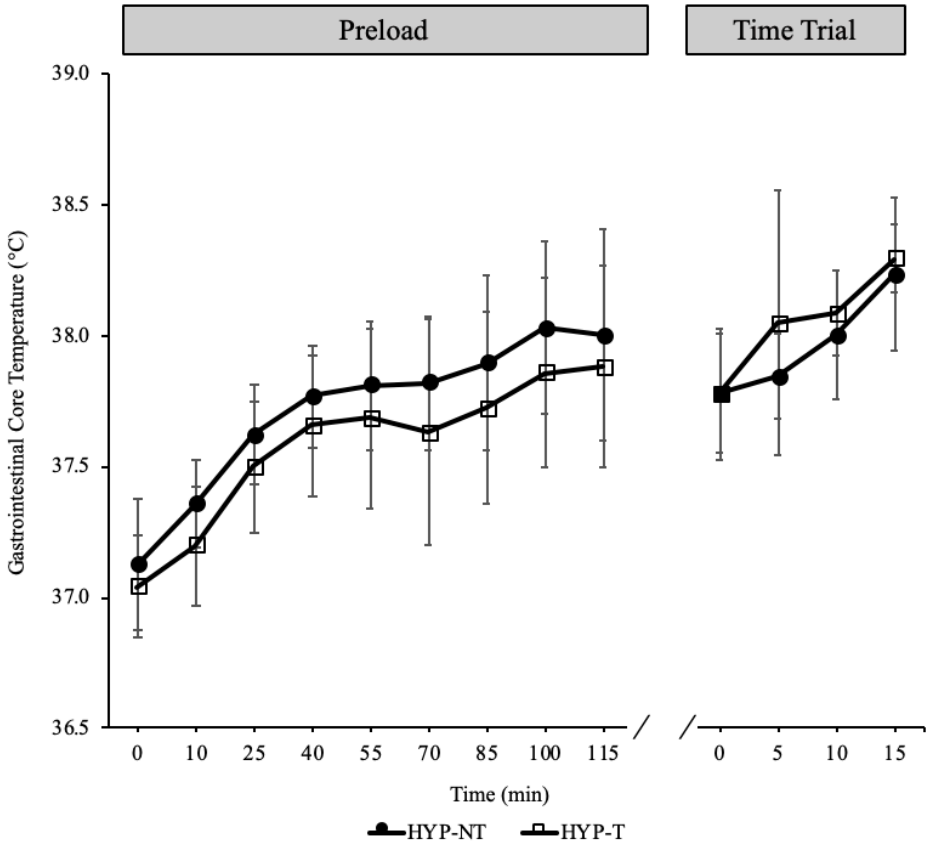

Figure 3.4. Gastrointestinal core temperature (T_{GI}) during the 120 min intermittent preload cycling exercise and during the 15 min time trial (TT) performance test in HYP-NT and HYP-T. Data are mean ± SD (n = 7).

3.3.4. Perceptual responses and VAS

Ratings of perceived exertion (RPE) increased throughout preload during both trials ($P < 0.001$) and was highest at the end of the preload in both. There was no main effect of trial ($P = 0.631$) nor interaction ($P = 0.426$) for RPE. Thermal comfort (TC) showed no main effect of time ($P = 0.083$), trial ($P = 0.575$) or interaction ($P = 0.584$). Participants rated a higher sensation of thirst during HYP-T compared to during HYP-NT ($P = 0.023$), which increased throughout both trials ($P < 0.001$) with a trial by time interaction effect ($P = 0.023$). During HYP-NT, participants did not rate any difference in thirst sensation from pre-preload to pre-TT ($P = 0.437$), compared to during HYP-T where participants rated a higher thirst sensation from pre-preload to pre-TT ($P < 0.001$). There were no differences in ratings of stomach fullness between trials ($P = 0.681$); however, participants ratings increased throughout experimental trials ($P = 0.010$) and there was a trial by time interaction effect ($P = 0.014$). During HYP-T, participants rated a lower sensation of stomach fullness from pre-preload to pre-TT ($P = 0.046$), which was not reported during HYP-NT ($P > 0.99$). Participants rated a higher level of bloating sensation during HYP-T ($P = 0.040$); however, there was no time ($P = 0.185$) or interaction effect ($P = 0.171$) (Figure 3.5).

3.3.5. Post-trial interview

During the exit interview, all seven participants believed it was a comparison of two drinks, two out of seven thought it was a comparison

of electrolytes vs. water, while the other five thought it was a comparison between different ratios of carbohydrate and protein drinks. No participant, therefore, indicated that they thought hydration status had been manipulated.

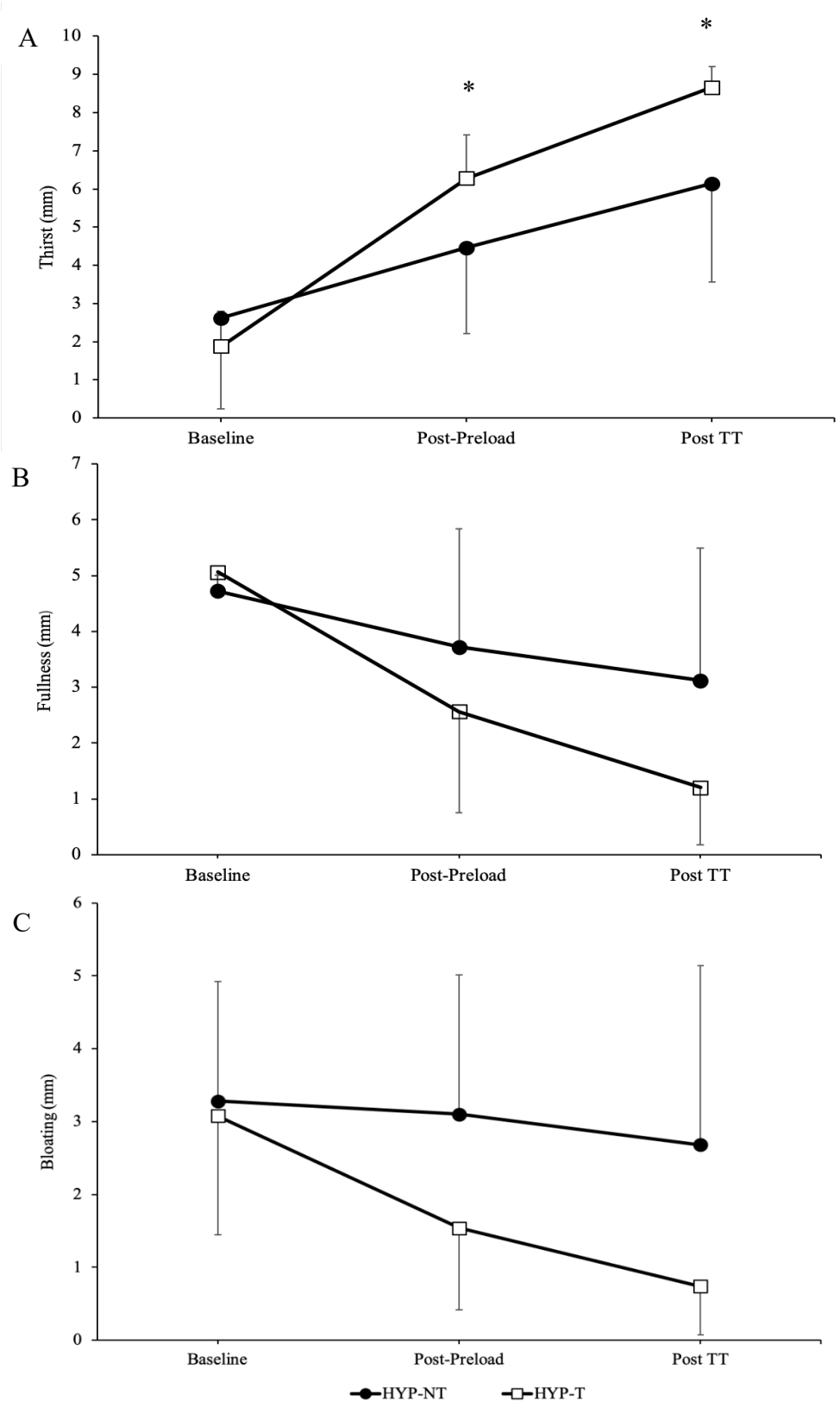

Figure 3.5. (A) Perceptual responses of thirst; (B) fullness and (C) bloating (mm) during HYP-NT and HYP-T, measured at baseline, post-preload and post TT. Data are mean ± SD (n = 7). *Significant ($P < 0.05$) difference between trials.

3.4. Discussion

The purpose of the present study was to investigate how perception and expectation of hydration status influences preloaded 15 min cycling TT performance in the heat (35 °C). The main finding of the present study was that perceived euhydration status was not associated with any differences in the amount of work completed during a 15 min TT. This finding suggests that the impairment in exercise performance when hypohydrated by ~2% is associated with the combined detrimental physiological and perceptual consequences of hypohydration, rather than the knowledge and expectation of hydration status.

In this study, we explored whether the impaired exercise performance, typically associated with prolonged, sub-maximal exercise in the heat, was altered by manipulation of perception and expectation of hydration status (i.e. by suggesting to participants that they were euhydrated, when they were not). It is important to emphasise that the aim of the present study was to induce the same magnitude of hypohydration in both experimental trials, which was evident as participants had a similar BM loss during the preload in both HYP-NT and HYP-T. As previous research has shown and supported in the current study, the progressive reduction in BM (by ~2%) resulted in similar elevations in T_{GI} and HR, which subsequently lead to a similar performance outcome in both trials. Cardiovascular strain was increased in both trials, demonstrated by the increase in HR reported from the second stage during the preload exercise. Previous evidence suggested

that due to alterations in body fluid content, which impairs the ability to dissipate heat via sweating, this elevation in HR likely occurs in response to the interaction between either the increase in core body or skin temperature and a decline in stroke volume (Cheung and McLellan, 1998; Trinity *et al.*, 2010). On the other hand, more recent findings suggest that the reduced stroke volume with hot skin temperature is due to an increase in HR (Chou *et al.*, 2019), likely in response to a reduced left-ventricular filling pressure that is associated with thermal stress (Wilson *et al.*, 2007). These cardiovascular adjustments, in combination with a reduction in PV from hypohydration, play a major contributing factor in potentially reducing blood flow to the muscle and skin which may limit exercise intensity shown by a decline in pacing and therefore, impairing subsequent exercise performance. An increase in T_{GI} was reported from the fourth stage of the preload, a typical response to occur, associated with exercise-induced hypohydration (González-Alonso *et al.*, 1997; James *et al.*, 2017; Funnell *et al.*, 2019). The increase in physiological strain was accompanied by a similar increase in perceptual strain during both experimental trials, excluding this as a potential influence on performance. The lack of difference in physiological and perceptual strain between trials suggests that knowledge of hydration status has no effect on the physiological or perceptual responses to exercise in the heat.

With perception influenced by not only physiological factors but by also psychological inputs (Baden *et al.*, 2005), the inclusion of a TT performance test in the present study, provided the opportunity to

investigate the association between perception and expectation of hydration status on the regulation of exercise intensity. In the present study, there were no differences in the pacing strategy adopted or the total work completed. A down regulation in pacing has been reported previously, where hypohydrated individuals decreased self-selected running speed throughout in accordance with an increased RPE (Stearns *et al.*, 2009). In contrast to this, no differences were observed in RPE between trials as the present study was not investigating the differences in actual hydration status but the interpretation of information. With the relationship between HR and RPE closely related (Zinoubi *et al.*, 2018) and without any physiological differences reported between trials, it is therefore, not surprising that no differences were observed in RPE between trials.

Despite no difference in thermal perceptions or perceived exertion, when participants were told that they were hypohydrated by 2%, they rated a higher sensation of thirst and felt less stomach bloating before the TT, compared to when told that they were euhydrated. The sensation of thirst has been proposed to play a key role in the anticipatory regulatory system by influencing motivation for exercise, whereby, individuals may decrease exercise intensity to prevent further fluid losses and before critically high core temperatures are attained (Noakes, 2007; Sawka and Noakes, 2007; Goulet, 2011). Contrary to these findings, the increase in sensation of thirst in the present study was not associated with any physiological or exercise

performance differences. Therefore, sensation of thirst was influenced by perception of hydration status.

In the present study, the exit interview revealed that the deception of hydration status was successful. As none of the seven participants believed the experimental protocol was to investigate the effects of perceived hydration status. Two out of seven thought it was a comparison of an electrolyte beverage and water, while the other five thought it was a comparison between different ratios of carbohydrate and protein drinks. The latter was the information provided to the participants upon the consent of participation in the present study. It is important to highlight that, in the present study, actual euhydration status was not investigated and, therefore, we are unable to compare the use of a nasogastric tube on hypohydration and euhydration states on exercise performance. However, both James *et al.* (2017) and Funnell *et al.* (2019) used a similar methodological approach conducted in the same laboratory, reporting cycling performance was impaired by ~8% to 11% when hypohydrated to ~2%. The exit interviews from these two studies, and the present study, support that the use of a nasogastric tube is an effective methodological approach to successfully blind participants from their true hydration status, while inducing the typical hypohydrated responses. A potential limitation to the present study was that the thermal strain induced during the preload was mild and did not result in differences in TC ratings from pre- to post-preload during HYP-T and HYP-NT. Therefore, potentially the exercise intensity was not intense enough or environmental temperatures were too

low to increase T_{GI} and perceptual ratings of thermal discomfort. Furthermore, information was not obtained on participants' previous experience of hypohydration and the inclusion of non-trained cyclists in the present study, where the knowledge, awareness and previous experience of the detrimental effects of hypohydration may have been limited compared to trained cyclists. Therefore, future research investigating the influence of perceived hydration status on cycling performance in the heat in trained cyclists is warranted.

3.5. Conclusion

In conclusion, perceived euhydration status did not result in a significantly greater amount of work completed during the preloaded 15 min TT performance test suggesting that the overtness of previous hypohydration literature did not produce confounding performance outcomes, which led to the rejection of H_1 and accepting of H_0. The present study demonstrated that the use of a nasogastric tube was an effective method to blind participants of their true hydration status during exercise. This methodological approach allowed the researchers to induce the typical physiological and perceptual consequences associated with hypohydration, without the awareness of doing so. Following on from the present study that investigated perceived hydration status on cycling performance, the next study investigated the other proposed acute intervention of pre- and per-cooling used in isolation and in combination on cycling performance in the heat in an attempt to alleviate thermal strain

and offset the detrimental effects of heat-induced decrements on performance outcomes.

Chapter 4. The Effects of Pre- and Per-Cooling Interventions on Cycling Performance in the Heat in Highly Trained Cyclists and Triathletes

4.1. Introduction

Prolonged exercise in hot and humid environmental conditions results in a marked physiological and perceptual strain, which can impair prolonged exercise capacity and endurance performance (Galloway and Maughan, 1997; Tucker *et al.*, 2004). As a result, several interventions have been investigated in an attempt to alleviate thermal strain and optimise exercise performance in thermally stressful environments. One such intervention involves cooling the body either before (pre-cooling) or during (per-cooling) exercise (Tyler, Sunderland and Cheung, 2015). Both cooling approaches can be effective (Bongers *et al.*, 2015); however, the mechanisms of benefit likely differ.

Pre-cooling is a popular intervention used in an attempt to offset the detrimental effects of heat stress on exercise performance by increasing heat storage capacity (Jones *et al.*, 2012) and, if effective, delaying the onset of heat-induced decrements in exercise performance (Marino, 2002). A number of pre-cooling interventions have been investigated and these are either applied externally (e.g. cold water immersion (CWI) or cooling vests), internally (e.g. ice slurry or cold drinks), or a mixed combination (Ross *et al.*, 2011; Stevens *et al.*, 2017). Whole-body CWI is the most effective intervention to reduce thermoregulatory strain prior to exercise (Booth, Marino and Ward, 1997; Choo *et al.*, 2018) but the reduced strain can be short-lived (<25 min) (Marino and Booth, 1998; Duffield *et al.*, 2010) and so per-cooling has been proposed as an additional or alternative approach.

Per-cooling has less of an effect on changing the physiological state of the individual (Bongers *et al.*, 2015; Bongers, Hopman and Eijsvogels, 2017); however, per-cooling can induce reductions in perceived strain and offset decrements to exercise performance (Ruddock *et al.*, 2017). A number of per-cooling techniques have been adopted and these include ice vests, ice slurry ingestion, and neck cooling collar. Ice vests appear to be a more effective approach as a larger surface area is cooled (Bongers *et al.*, 2015) than compared to cooling the neck region; however, the practicality of ice vests as a per-cooling method is limited during sports such as cycling due to the mass of the vests and accessibility to the torso, and so neck cooling may be more practical. The neck region has a high magnitude of alliesthesial thermosensitivity, due to the proximity of large blood vessels to the skin in this area (Shvartz, 1976; Cotter and Taylor, 2005) and cooling the neck during exercise in the heat can improve exercise performance by ~6% (Tyler and Sunderland, 2008, 2011a, 2011b; Tyler, Wild and Sunderland, 2010).

With performance impairments offset by the application of pre- and per-cooling (Bongers *et al.*, 2015; Bongers, Hopman and Eijsvogels, 2017) in isolation, it appears logical to suggest that a combined approach would be more effective; however, Schulze *et al.* (2015) and Stevens *et al.* (2017) reported that pre- and per-cooling interventions were equally beneficial to performance when used in combination or in isolation. Mixed internal (Schulze *et al.*, 2015; Stevens *et al.*, 2017) and external (Stevens *et al.*, 2017) pre-cooling interventions followed by internal ice slurry and menthol per-cooling via ice slurry and menthol ingestion or rinsing have been investigated

(Riera *et al.*, 2014; Schulze *et al.*, 2015; Stevens *et al.*, 2017). In one such study, combined external pre-cooling (CWI) with internal per-cooling (ice slurry) had no additional benefit to performance compared to when per-cooling was used in isolation (Stevens *et al.*, 2017). Internal pre- and per-cooling effectively alters the perception of thermal strain, with or without actual physiological change, but the ingestion of large volumes of an ice slurry may be impractical as it can lead to higher gastrointestinal discomfort (Stevens *et al.*, 2016, 2017). This may limit the performance benefits of cooling especially during sports that involve high-intensity exercise (Ross *et al.*, 2011; Stevens *et al.*, 2016). External cooling would avoid such gastrointestinal discomfort but currently no evidence exists on the effect of external pre-cooling and external per-cooling used in combination on exercise performance. Therefore, the aim of the present study, was to investigate the effect of isolated and combined pre- and per-external cooling methods on cycling performance in the heat in well-trained athletes.

It was hypothesised that:

H_0: neither isolated nor combined pre- and per-cooling interventions would increase distance covered during 15 min time trial (TT) cycling performance.

H_1: isolated and combined pre- and per-cooling interventions would increase distance cycled during 15 min TT by sustaining a higher self-selected power output during the TT with performance improvements seen during the latter

stages of the TT (pre-cooling by reducing physiological and perceptual strain and per-cooling by reducing the perceived thermal strain).

H_2: combined pre- and per-cooling would be the most effective approach as a result of a cumulative and longer-lasting benefit.

4.2. Methods

4.2.1. Participants

Nine, highly-trained, non-heat acclimated male cyclist/triathletes (mean ± SD: age 32.1 ± 10.3 y, stature 183.5 ± 6.5 cm, body mass 75.8 ± 8.9 kg, body fat percentage 6 ± 4.7% and peak oxygen uptake ($\dot{V}O_{2peak}$) 65 ± 7 $mL \cdot min^{-1} \cdot kg^{-1}$) participated. All participants completed a health screening questionnaire and received written details outlining the nature of the study and any risks and discomforts associated with taking part, before giving their written, fully-informed consent to participate. This study was approved by the University of Roehampton's ethics committee (LSC 17/208, see Appendix B3) and all procedures and protocols adhered to the guidelines of the World Medical Association (*Declaration of Helsinki*). For calibration measurements of equipment used see Appendix E1.

4.2.2. Experimental design (Figure 4.1)

Participants visited the laboratory on five occasions for one preliminary trial and four experimental trials. Each experimental trial required participants to cycle in the heat (40 ºC, 50% relative humidity (rh)) for 45

min at 50% peak power output (PPO) before completing a 15 min time trial (TT) in one of four conditions:

1. No cooling (CON)
2. Per-cooling only (PER)
3. Pre-cooling only (PRE)
4. Pre- and per-cooling in combination (PRE+PER)

The four experimental trials were conducted in a randomized, counter-balanced order, separated by 7 days to 14 days, and performed at the same time of day (± 1 h) to minimise the effects of circadian rhythm on heart rate (HR) and rectal temperature (T_{re}). Participants kept a food diary 24 h before the first trial and repeated the same diet before all subsequent experimental trials. Participants refrained from strenuous activity and, alcohol and caffeine intake, 48 h and 24 h prior to each experimental trial, respectively. The exercise duration and format with a fixed intensity preload and 15 min TT was chosen to mimic a cycling race, whereby cyclists remain in a peloton together before the break away for the final sprint finish. A familiarised 15 min TT preloaded with 45 min of fixed intensity cycling has been previously shown to be highly reliable in hot environmental conditions (CV 3.6%) (Che Jusoh *et al.*, 2015).

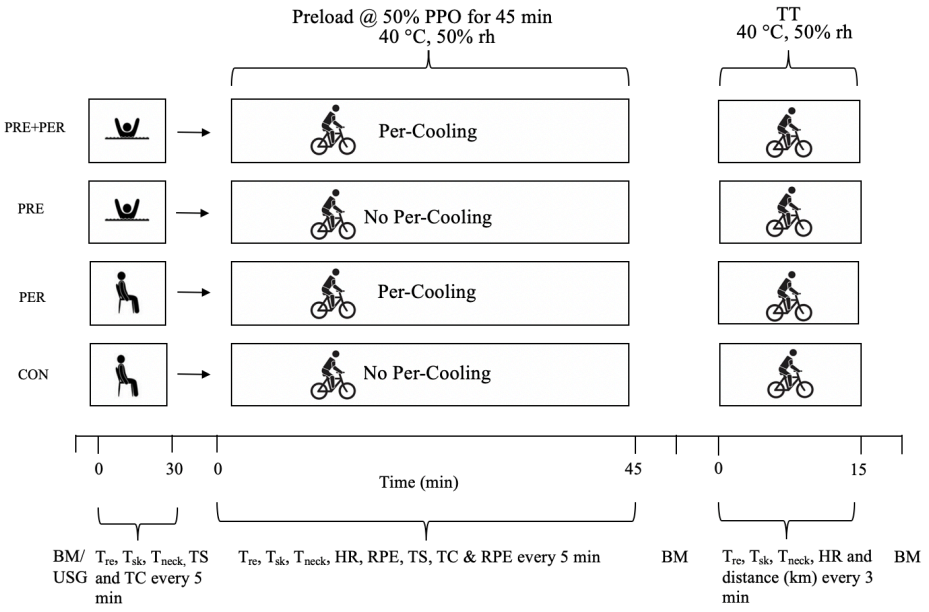

Figure 4.1. Schematic layout of the experimental protocols. Participants completed four experimental trials. Two involved pre-cooling via cold water immersion (CWI) and two involved seated rest in ambient laboratory conditions before participants entered the controlled environment (40 °C, 50% rh) and cycled for 45 min at 50% peak power output (PPO). On completion of the preload, a 15 min time trial (TT) was completed.

4.2.3. Preliminary testing

Stature (Harpenden Stadiometer, Holtain Ltd, UK) and body mass (Seca, Birmingham, UK) were recorded before $\dot{V}O_{2peak}$ and PPO were determined in ambient laboratory conditions (21 ± 1 °C and 55 ± 4% rh) using an incremental exercise test (starting at 95 watts (W) and increasing by 35 W every 3 min) until volitional exhaustion, on an electromagnetically braked cycle ergometer (Lode Excalibur Sport, Groningen, Netherlands). Breath-by-breath gas exchange was continuously measured using an online metabolic cart (Oxycon Pro Jaeger, Germany) with $\dot{V}O_{2peak}$ defined as the highest value, using a rolling 5 breath average. On completion of the exercise test, participants rested for 15 min before they entered a controlled

environment (Weiss, Technik, UK) and were familiarised with the CON experimental trial. During the familiarisation, participants completed the 45 min preload set at 50% PPO followed by the 15 min TT (40 °C, 50% rh). Sweat losses were determined from changes in body mass accounting for urine production and fluid consumption.

4.2.4. Experimental trials

Upon arrival, a mid-flow urine sample was provided for the measurement of urine specific gravity (USG), using a hand-held pen refractometer (Atago, pen refractometer, PEN-Urine S.G, Tokyo, Japan). All participants reported to the laboratory euhydrated (USG < 1.020). Following this, participants self-recorded nude body mass and self-inserted a rectal thermistor (REC-U-VL3-0, Grant Instruments (Cambridge) Ltd., UK) ~10 cm past the anal sphincter before affixing a HR monitor (Polar Electro Ltd., Kempele, Finland) to their upper torso. The rectal thermistor was connected to a portable data logger (Squirrel 2020 Series, Grant Instruments (Cambridge) Ltd., UK). Skin temperature and mean neck temperature (T_{neck}) were recorded continuously using wireless thermochron iButton skin temperature data loggers (DS1922L, Thermochron iButton, USA). iButtons were attached using transparent adhesive dressing (Tegaderm, 3M Health Care, St Paul, MN) and waterproof tape (Transpore, 3M Health Care, St Paul, MN) to the sternal notch (T_{sn}), forearm (T_f), thigh (T_t) & calf muscle (T_c) on the right side of the body and on the left and right side of the neck. Mean-weighted skin temperature (T_{sk}) was calculated using the equation of

Ramanathan (T_{sk} = ([0.3 x T_{sn}] + [0.3 x T_f]) + [0.2 x T_t] + [0.2 x T_c]) (Ramanathan, 1964) and T_{neck} was calculated as the mean temperature from two locations on the neck.

Following a 30 min period of pre-cooling (see 4.2.5) or seated rest, participants entered the controlled environment and cycled for 45 min at 50% PPO (177 ± 12 W) with 1.5 $m.s^{-1}$ of airflow to the face. Water ingestion was matched to the individual sweat rates measured during the familiarisation trial. The volume of fluid provided during the preload was divided into ten equal aliquots (79.9 ± 9.5 mL), provided at 5 min intervals, and was stored in the environmental temperature (40 °C) to prevent unintentional internal cooling. T_{re}, T_{sk}, T_{neck}, and HR, ratings of perceived exertion (RPE; Borg, 1982), thermal sensation (TS; Young *et al.*, 1987), and thermal comfort (TC; Gagge, Stolwijk and Hardy, 1967) were recorded and rated every 5 min. On completion of the preload, participants towel-dried and were weighed nude inside the environmental chamber before returning to the cycle ergometer to complete the TT.

For the TT, power output was initially set at 90% PPO (319 ± 23 W) and participants could increase or decrease the workload as desired by pressing the up or down button on the ergometer's console. Participants were provided with standardised verbal instructions to cover as much distance (km) as possible during the 15 min and the only feedback provided was the time remaining. No per-cooling or motivation was given to the participants during the TT. Participants were able to drink ad libitum. T_{re},

T_{sk}, T_{neck}, HR, and the distance (km) completed were recorded at 3 min intervals. Perceptual data were not collected during the TT to avoid disturbing the participants. On completion of the TT, participants exited the controlled environment, towel dried and self-recorded nude body mass, which we calculated sweat losses taking into account voluntary fluid consumed during TT.

4.2.5. Cooling interventions

During the pre-cooling trials (PRE and PRE+PER), participants sat in an inflatable water-immersion pool (Physique, RecoveryTub Team, UK), wearing swim wear with cool water (24 ºC reduced to 22 ºC during the immersion period) up to their neck for 30 min, while consuming a total of 7.5 mL·kg^{-1} of cold water (~10 ºC) (1.25 mL·kg^{-1} every 5 min). This cold water immersion protocol was modified from previous literature (Marino and Booth, 1998; Quod *et al.*, 2008) and adjusted after piloting. Thermal perceptions (TS and TC) were rated and T_{re}, T_{sk}, T_{neck} were recorded every 5 min. Participants had ~5 min to towel dry and put on cycling kit before entering the controlled environment. During non-pre-cooling trials (CON and PER), participants rested in a seated position in ambient laboratory conditions while consuming 7.5 mL·kg^{-1} of warm water (~37 ºC) at the same rate.

During the per-cooling trials (PER and PRE+PER), participants completed the 45 min preload while wearing a neck cooling collar (CCX; Black Ice LLC, Lakeland, TN), that was replaced at 15 min intervals to maintain the

cooling stimulus, established from piloting and based on equipment availability. No per-cooling was applied during 15 min TT. The neck collar had five compartments that were drained of the Black Ice cooling reagent and filled with approximately 120 g of gel refrigerant (BDH Laboratory Supplies, Poole, United Kingdom), as per previous research (e.g. Tyler and Sunderland, 2011a).

4.2.6. Statistical analyses

Data were analysed using SPSS (version 26, SPSS Inc.). Parametric assumptions were met unless stated otherwise. A one-way ANOVA was conducted to evaluate differences between trials for the distance cycled, and a two-way repeated measures ANOVA was performed to evaluate differences between trials and time for pacing, thermoregulatory, cardiovascular and perceptual variables. Where the assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimate. Where significant outcomes were present, post-hoc tests with Bonferroni corrections were performed. The alpha level was $P < 0.05$. Data are presented as mean ± SD.

4.3. Results

4.3.1. Time trial (TT): Physiological response and performance data

There were main effects of time for T_{re}, T_{sk} and HR, all increasing progressively throughout the TT (all $P < 0.001$). There were no effects of time on T_{neck} ($P = 0.078$). There were no main trial or interaction effects for T_{re}, T_{sk}, T_{neck} and HR (all $P > 0.05$).

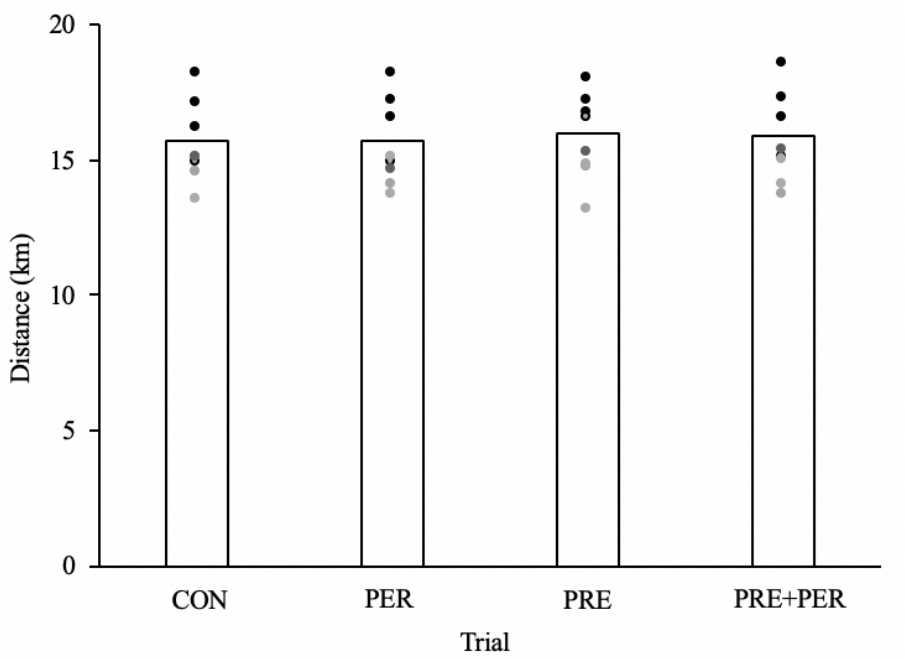

Figure 4.2. The mean distance (km) cycled during 15 min TT performance test during the four experimental trials (CON, PER, PRE and PRE+PER) (n = 9).

Participants covered a similar distance in all trials (CON: 15.7 ± 1.6 km; PER: 15.7 ± 1.5 km; PRE: 15.9 ± 1.5 km; PRE+PER: 15.8 ± 1.5 km, $P = 0.773$) presented in Figure 4.2. TT pacing was also similar with no differences in the distance (km) completed between or within trials (all $P > 0.05$).

4.3.2. Pre-exercise: Physiological and perceptual data

Pre-immersion measurements of T_{re}, T_{sk}, T_{neck}, and thermal perceptions (TC and TS) were not different between trials (all $P > 0.05$), indicating participants started each trial in a similar physiological and perceptual state.

There were main effects of trial and interaction for T_{re}, T_{sk}, TC and TS (all $P < 0.001$) but not for T_{neck} ($P = 0.813$; $P = 0.214$) during CWI. There was a main effect of time for T_{re}, TC and TS (all $P < 0.001$) where T_{re} (Figure 4.3) progressively decreased and participants rated themselves more uncomfortable and cooler throughout CWI. There were no main effects of time reported for T_{sk} (Figure 4.5A) ($P = 0.869$) or T_{neck} (Figure 4.5B) ($P = 0.095$). During CWI, T_{re} and T_{sk} were lower from 20 min and 15 min, respectively during PRE and PRE+PER when compared to CON and PER (all $P < 0.05$) and remained lower until the end of CWI. TC and TS were lower during PRE and PRE+PER compared to CON and PER during CWI (all $P < 0.001$), with no differences between non-pre-cooling trials (CON, PER) throughout the 30 min resting period (all $P > 0.99$).

Upon exiting the water and starting the preload (~10 min), T_{re} decreased further by 0.38 ± 0.55 °C and 0.22 ± 0.42 °C, leading to a total reduction in T_{re} of 1.19 ± 0.71 °C and 1.00 ± 0.48 °C in PRE and PRE+PER, respectively. While T_{sk} increased by 3.19 ± 1.83 °C and 2.73 ± 1.08 °C in PRE and PRE+PER, respectively. The reduction in T_{re} was similar in PRE and PRE+PER (P = 0.546) and no differences between PRE and PRE+PER in the increase in T_{sk} (P = 0.520).

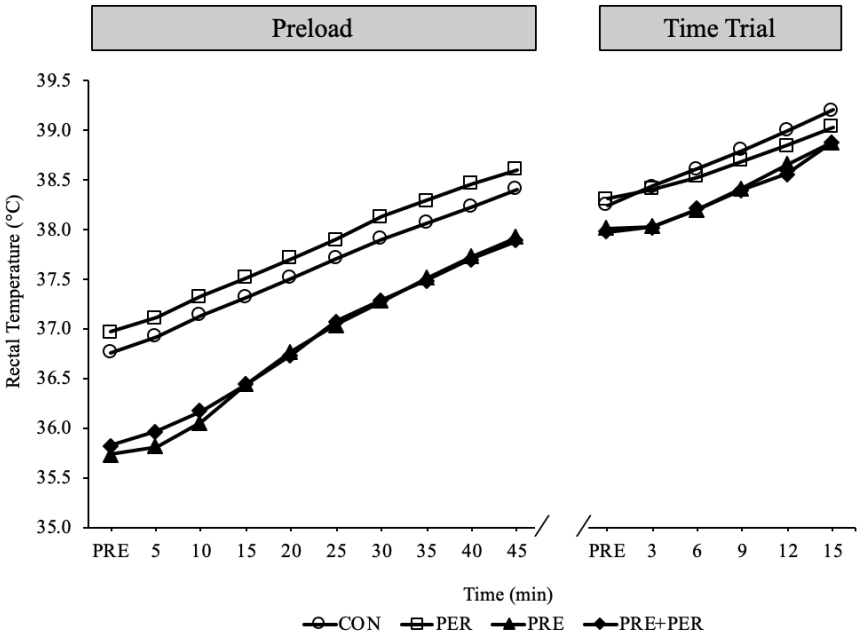

Figure 4.3. Mean rectal temperature (ºC) during 45 min preload exercise and during 15 min TT performance test in the control (CON), per-cooling (PER), pre-cooling without per-cooling (PRE) and pre-cooling with per-cooling (PRE+PER) (n = 9).

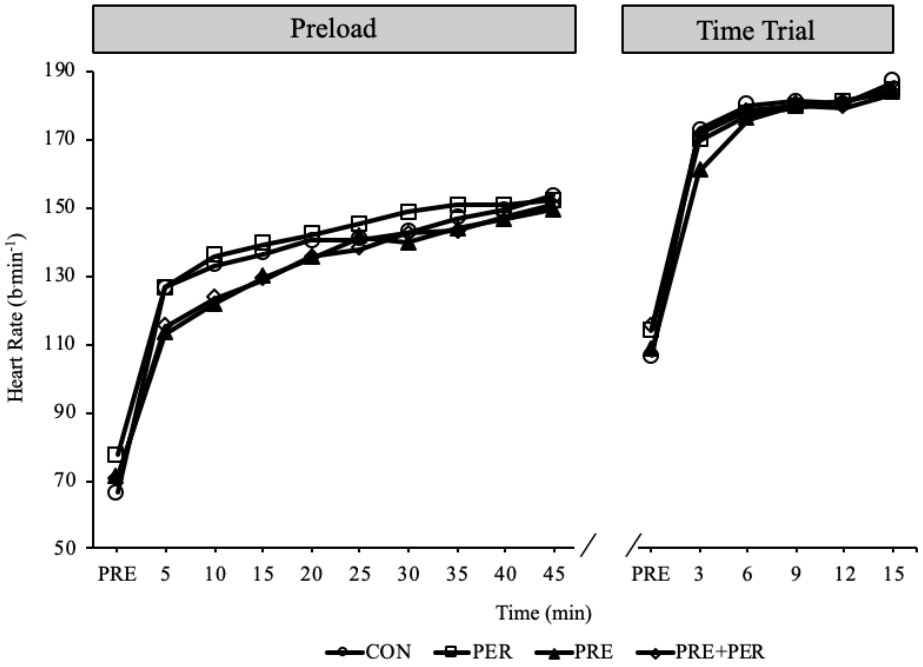

Figure 4.4. Mean heart rate (b.min^{-1}) response during 45 min preload exercise and during the 15 min TT performance test in the control (CON), per-cooling (PER), pre-cooling without per-cooling (PRE) and pre-cooling with per-cooling (PRE+PER) (n = 9).

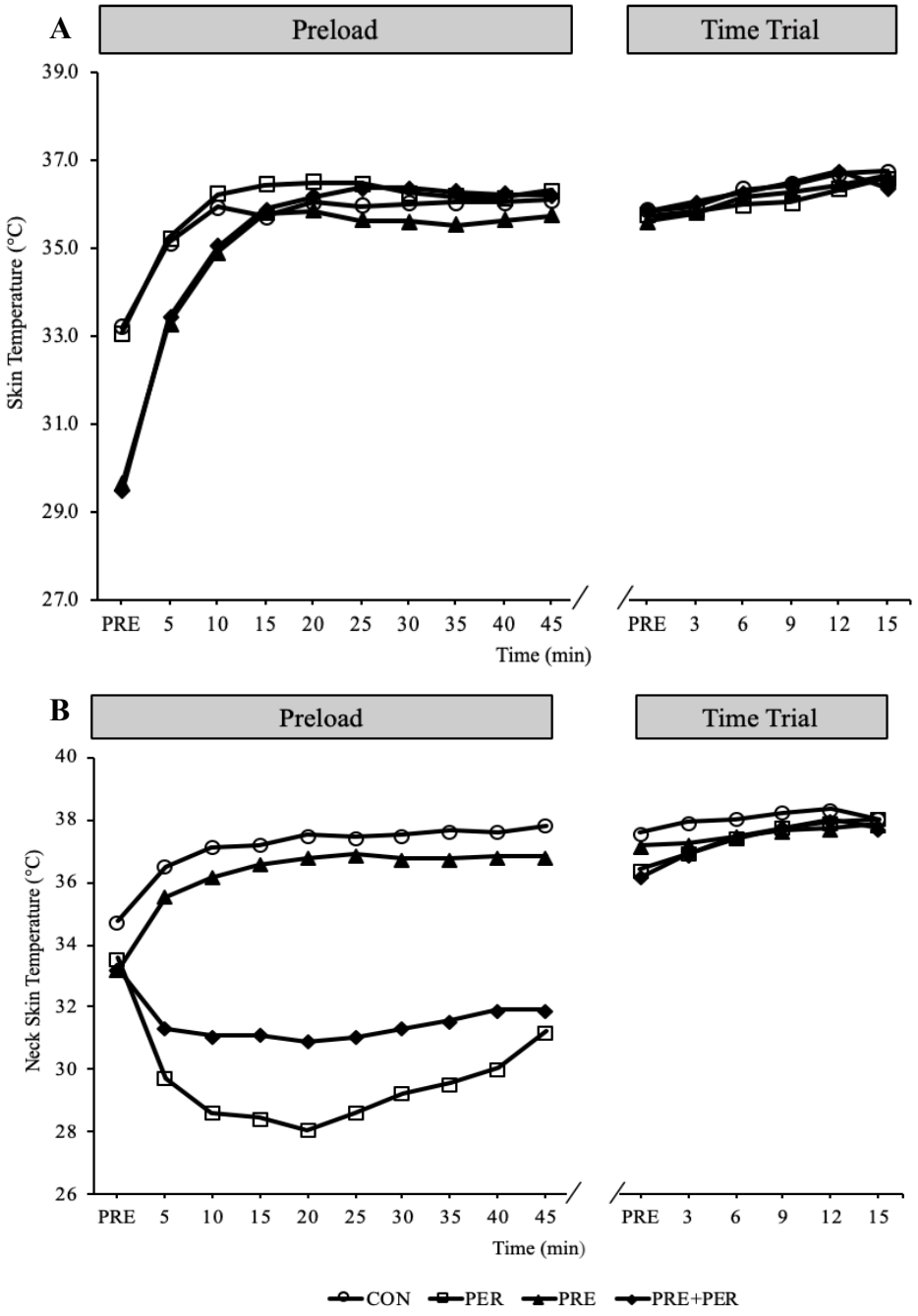

Figure 4.5. (A) Mean skin temperature (°C) (B) Mean neck skin temperature (°C) during 45 min preload and during the 15 min TT performance test in the control (CON), per-cooling (PER), pre-cooling without per-cooling (PRE) and pre-cooling with per-cooling (PRE+PER) (n = 9).

4.3.3. Preload: Physiological data (Table 4.1)

There were main effects of trial (all $P < 0.002$), time (all $P < 0.05$) with all increasing progressively throughout the preload and an interaction effect (all $P < 0.05$) for T_{re}, T_{sk}, T_{neck} and HR.

At the start of the preload, T_{re} and T_{sk} were lower in PRE and PRE+PER, compared to CON and PER (all $P < 0.05$), with T_{neck} and HR similar between all trials (all $P > 0.05$). No differences were revealed in T_{re}, T_{sk}, T_{neck}, and HR between non pre-cooling trials (all $P > 0.99$) or between pre-cooling trials (all $P > 0.99$).

During the preload, T_{re} remained lower in PRE+PER compared to CON ($P = 0.010$) and PER ($P = 0.019$). T_{re} remained lower in PRE compared to CON ($P = 0.003$) throughout but was only lower for the first 20 min when compared to PER ($P = 0.025$) as from 25 min into the preload no differences were observed ($P = 0.051$) (Figure 4.3). The rates at which T_{re} increased was similar between all trials (CON and PER: 0.04 ± 0.01 °C.min^{-1}; PRE and PRE+PER: 0.05 ± 0.01 °C.min^{-1}, $P = 0.061$), with all increasing progressively throughout the preload (all $P < 0.001$). T_{sk} was lower for the first 15 min of preload during pre-cooling trials compared to non-pre-cooling trials (all $P < 0.05$), with no differences revealed thereafter (all $P > 0.05$), and T_{sk} increased progressively throughout the trial (CON: +3.2 ± 1.0 °C; PER: +3.2 ± 1.3 °C; PRE: +6.8 ± 1.8 °C; PRE+PER: +6.9 ± 2.3 °C) (Figure 4.5A). T_{neck} was lower during PER and PER+PRE compared to CON and PRE (all

P < 0.001). T_{neck} progressively increased during non per-cooling trials (CON: 3.0 ± 0.6 ºC; PRE: 3.5 ± 1.4 ºC) but decreased in PER (-3.6 ± 2.3 °C) and PRE+PER (-2.9 ± 2.4 °C) (Figure 4.5B). HR was lower for the first 10 min in both PRE and PRE+PER compared to CON (P = 0.011, P = 0.008) and lower between 10 min and 15 min in PRE and PRE+PER compared to PER (P = 0.010, P < 0.001) (Figure 4.3). HR was similar between both non pre-cooling (all P > 0.05) and between both pre-cooling trials (all P > 0.05), with HR increasing progressively throughout each trial (all P < 0.001).

At the end of the preload, T_{re} was lower in PRE+PER compared to CON (P = 0.010) and PER (P = 0.019), with T_{re} lower in PRE compared to CON (P = 0.003) but similar to PER (P = 0.054) (Table 4.1). T_{neck} was lower in PER and PRE+PER compared to CON and PRE (all P < 0.01).T_{sk} and HR was similar between all trials (all P > 0.99) and no differences between trials were revealed in total sweat losses during preload ~1.37 ± 0.24 L (all P > 0.05).

4.3.4. Preload: Perceptual data

There were main effects of trial for TS (P = 0.016) and RPE (P = 0.024) but not for TC (P = 0.326). During the preload, there was an effect of time (all P < 0.001) where all increased progressively, however there was no interaction effect for TS, TC and RPE (all P > 0.05).

At the start the preload, participants rated similar levels of TS and TC in all trials (all P > 0.05), data are presented in Table 4.1. During the preload mean TS was lower in PRE+PER compared to CON (P = 0.048) with no

differences between all other trials (all $P > 0.05$). Participants rated similar TC between trials (all $P > 0.05$) and RPE (all $P > 0.05$). At the end of the preload trial, participants rated a similar TS (all $P > 0.99$), TC (all $P > 0.05$) and RPE (all $P > 0.05$).

Table 4.1. Mean ± SD for rectal temperature (T_{re}), heart rate (HR), thermal sensation (TS), thermal comfort (TC) and ratings of perceived exertion (RPE) before and after preload and rectal temperature (T_{re}), heart rate (HR) before and after 15 min time trial (TT) performance test during all trials (n = 9). *Significant ($P < 0.05$) difference compared to CON.

		45 min Preload					15 min TT		
Time	**Trial**	**T_{re} (°C)**	**HR (b.min $^{-1}$)**	**TC**	**TS**	**RPE**	**Time**	**T_{re} (°C)**	**HR (b.min $^{-1}$)**
Start	CON	36.76 ± 0.40	66 ± 6	1 ± 0.0	4 ± 0.7	-	Start	38.25 ± 0.55	106 ± 12
	PER	36.97 ± 0.32	77 ± 11	1 ± 0.5	5 ± 0.7	-		38.31 ± 0.33	114 ± 15
	PRE	35.74 ± 0.74*	71 ± 14	1 ± 0.9	4 ± 1.3	-		38.01 ± 0.43	108 ± 15
	PRE+PER	35.84 ± 0.58*	70 ± 12	1 ± 0.4	4 ± 0.9	-		37.98 ± 0.39	115 ± 11
Finish	CON	38.41 ± 0.53	153 ± 16	3 ± 0.5	6 ± 0.4	12 ± 0.9	Finish	39.20 ± 0.52	187 ± 9
	PER	38.61 ± 0.33	152 ± 17	3 ± 0.6	6 ± 0.4	12 ± 0.8		39.04 ± 0.34	184 ± 9
	PRE	37.92 ± 0.35*	149 ± 15	3 ± 0.9	6 ± 0.7	12 ± 0.8		38.88 ± 0.47	184 ± 9
	PRE+PER	37.89 ± 0.37*	151 ± 10	3 ± 0.7	6 ± 0.7	12 ± 1.0		38.88 ± 0.48	183 ± 9

4.4. Discussion

In the present study, the use of external pre-cooling in isolation and in combination with external per-cooling was effective at reducing markers of physiological strain for a limited time during the preload, however, by the time the participants stared the TT these responses had worn off. The main findings in the present study therefore reject the two research hypotheses (H_1 & H_2) that neither isolated nor combined external pre- and per-cooling in highly trained endurance athletes improved 15 min cycling TT performance in the heat (40 °C).

The mechanisms that underpin reduced exercise performance under heat stress are multi-factorial and interlinked with the attainment of a high core body temperature, limiting an individual's capacity to exercise in the heat (Nielsen *et al.*, 1993; González-Alonso *et al.*, 1999). Therefore, the theoretical basis of pre-cooling is to delay the onset of hyperthermia induced decrements in exercise performance by lowering initial starting rectal temperature and widening the margin to store heat. This would, in theory, offset the reductions in cycling performance and allow more work to be completed by delaying the anticipatory adjustments in work rate, a protective phenomenon hypothesised to occur to enable exercise to be completed within homeostatic limits of the body (Quod, Martin and Laursen, 2006). The data in the present study showed that T_{re} was lower during the preload with isolated (PRE) and combined cooling (PRE+PER) compared to when no pre-

cooling was applied, but this response was not seen to last for greater than 45 min.

It was hypothesised (H_2) that combining per-cooling following pre-cooling would offer a cumulative benefit where thermal strain during exercise would be much higher than compared to rest. Consequently, in the present study the addition of per-cooling was not shown to add any additional advantage received from CWI in lowering perceived thermal strain. This outcome was unsurprising as the neck region only constitutes ~1% of the body's surface area (Tyler and Sunderland, 2011a) and so cooling such a small surface area was not shown change the physiological state of an individual in the present study and in previous neck cooling collar studies (Tyler and Sunderland, 2011b). Per-cooling has been shown to reduce perceptual strain (Ruddock *et al.*, 2017); however, in the present study perceived exertion and thermal state were not reduced with the addition of per-cooling even though T_{re} was reduced. The data in the present study, and others (Schlader *et al.*, 2011b; Schulze *et al.*, 2015), provide evidence to support that perception of thermal strain may be the key regulator of power output rather than actual thermal strain per se. In previous combined cooling studies, exercise performance was improved in the absence of a reduced T_{sk} or T_{re} but when perceived thermal strain was alleviated, demonstrating that the stimulation of thermal behaviours are a key regulator of performance in the heat (Minniti, Tyler and Sunderland, 2011; Schulze *et al.*, 2015).

In the present study, the inclusion of a preload trial provided the opportunity to investigate the physiological and perceptual responses to the cooling interventions used during steady-state exercise, whereas the self-paced performance test assessed thermoregulatory behaviour. A rapid rewarming of the skin was observed to occur within 10 min of the preload, likely explained by the high thermal load from the environmental temperatures used within the study. As previously reported by Schlader *et al*. (2011b) this peripheral thermal input may have taken precedent over actual core body temperature and subsequently influenced perceived thermal strain, as participants rated the same level of thermal comfort and sensation, regardless of cooling intervention. On the other hand, perceived thermal strain has been found to be lower during other neck cooling collar studies without differences in core temperature, while unlikely, we are unable to conclude if this was due to any difference in skin temperature as this was not measured in these studies (Tyler, Wild and Sunderland, 2010; Tyler and Sunderland, 2011a, 2011b). With ambient air temperature a primary determinant for both skin and thermal temperature gradient, the use of a higher ambient temperature of 40 °C in the present study might explain the early dissipating benefits of the cooling intervention by limiting convective heat transfer and therefore, increasing rectal temperature in contrast to the lower ambient air temperatures used in previous cooling studies (Tyler, Wild and Sunderland, 2010; Tyler and Sunderland, 2011b). As well as perceived thermal strain, no differences were reported in perceived exertion. There is evidence to suggest that independent of rectal temperature or cardiovascular adjustments but with

high skin temperature (Tatterson *et al.*, 2000; Tucker *et al.*, 2006) self-paced exercise has been downregulated. Furthermore, the data from the present study further supports that thermal behaviour is a key regulator for performance in the heat as participants experienced similar levels of thermal strain and completed a similar distance during the performance test.

Potential explanations for the lack of a performance benefit reported in the present study compared to previous studies include the lack of thermoregulatory strain imposed upon the participant during the preload may have negated the beneficial effects of the cooling intervention investigated. In addition to this potential limitation the inclusion of high aerobic fitness levels of the participants, the use of airflow during trials, and the performance test adopted. Fitter individuals through repeated physical training gain some thermal adaptations and therefore, may have an increased tolerance to the heat regardless of experimental manipulations (Cheung and McLellan, 1998) and so smaller decrements are seen in performance outcomes (Ely *et al.*, 2008). Another potential reason is the provision of convective cooling during the trial – something often lacking in the pre- and per-cooling literature. Evidence exists to support that pre-cooling has minor benefit on performance when airflow is present, however, pre-cooling is more effective without airflow than with it (Morrison, Cheung and Cotter, 2014). Providing convective cooling in addition to conductive cooling may help to explain why no benefit in performance was observed in the present study and the ecological validity of previous pre- and per-cooling studies

should be questioned, as still wind conditions do not accurately replicate the thermal environment encountered during athletic competitions (Morrison, Cheung and Cotter, 2014). Finally, a 15 min TT performance test preloaded with a 45 min fixed intensity cycling exercise in highly trained individuals under thermal strain, has been demonstrated to be a highly reliable test (coefficient of variance of 3.6%) (Che Jusoh *et al.*, 2015); however, it may lack the sensitivity to detect small but meaningful changings in performance that may have occurred in the present study (Currell and Jeukendrup, 2008). Performance improvements following pre-cooling have often been reported to occur during the final 10 min of a 40 min performance test (Duffield *et al.*, 2010) or during the last 20% of a 40 km TT (Tucker *et al.*, 2004) and therefore, for pre-cooling to be of benefit the performance test may need to be longer in duration. Future studies should investigate whether the manipulation of both rectal and skin temperature through the use of pre- and per-cooling interventions can provide a performance benefit in highly trained individuals, when exercising in extreme hot environmental conditions (>40 °C).

4.5. Conclusion

In conclusion, neither pre- nor per-cooling, in isolation or combination, improved 15 min cycling performance in 40 °C, therefore, the present study accepts H_0 and rejects both H_1 and H_2. The lack of performance improvement with external isolated and external combined cooling was likely due to the minimal physiological changes and an absence of differences in thermal

perceptions or perceived exertion observed. The next study addresses the chronic intervention known as HA on its response to changing the physiological and perceptual state of the individual when exercising under heat stress.

Chapter 5. **Short-term Isothermic Heat Acclimation Elicits Beneficial Adaptations but Medium-term Elicits a more Complete Adaptation**

5.1. Introduction

Exercising for a prolonged duration in a thermally stressful environment places the body under greater physiological and perceptual strain than when exercising in temperate conditions (Galloway and Maughan, 1997; Tucker *et al.*, 2004). The greater strain often results in reduced aerobic exercise performance (Ely *et al.*, 2008) and may even result in serious heat illness, such as heat exhaustion and heat stroke (Wendt, Van Loon and Van Marken Lichtenbelt, 2007). Heat acclimation (HA) has been proposed as one of the most effective interventions to be incorporated into an athlete's training programme to reduce physiological strain and improve exercise performance in hot environmental conditions (Racinais *et al.*, 2015; Tyler *et al.*, 2016). Currently the optimal HA protocol is still unknown despite a large body of research manipulating the intensity and duration of exercise, the frequency of HA exposures, and the type of HA used (Taylor and Cotter, 2006).

Repeated exposure to thermal stress can induce beneficial adaptations that include a reduced body temperature (Garrett, Rehrer and Patterson, 2011), increases in sweating sensitivity and rate (Lorenzo and Minson, 2010; Buono *et al.*, 2018), improved cardiovascular stability (Frank *et al.*, 2001; Périard *et al.*, 2016), lower perceptual strain, and improved exercise economy (Tyler *et al.*, 2016). Up to 80% of adaptations occur in the first 4 days to 7 days of exposure (short-term heat acclimation (STHA)); however, the magnitude of adaptation appears to be greater when medium-

term (MTHA; 7 days to 14 days) and long-term (LTHA; > 15 days) HA protocols are used (Pandolf, 1998; Tyler *et al.*, 2016) and not all adaptations occur over the same time course. For example, heart rate adaptations typically occur well before improvements in performance and the sudomotor responses are observed (Armstrong and Maresh, 1991; Périard *et al.*, 2016). For thermal adaptations to occur, heat stress must induce physiological strain of a magnitude above an adaptation threshold (Taylor, 2014) and the magnitude of the adaptation appears dependent on the extent and frequency of the thermal strain and thermal impulse provided. It has been proposed that the threshold for adaptation may be the attainment and maintenance of a rectal temperature of ~38.5 °C (Gibson *et al.*, 2015) because at this temperature sudomotor and vasomotor thermoeffector responses are challenged (Fox *et al.*, 1964) and heat shock proteins are expressed (Gibson *et al.*, 2015). It may be difficult to reach and maintain such an internal temperature using traditional constant work HA protocols, but a controlled isothermic HA protocol overcomes this issue by ensuring that the target rectal temperature is reached through exercise and then maintained using passive (e.g. resting) and active (e.g. exercise) heat stress.

Another potential practical benefit of isothermic HA protocols is that thermal adaptations may be achieved with shorter exercise durations and lower exercise intensities than fixed intensity HA protocols (Gibson *et al.*, 2015) and therefore, they may be appropriate during the tapering phase in

training (Tyler *et al.*, 2016). Thermal adaptations are lost at a rate of approximately 2.5% per day when individuals are not exposed to heat and so HA should be undertaken as close to competition as possible to minimise de-acclimation (Daanen, Racinais and Périard, 2018); however, an overly exerting HA protocol may compromise subsequent exercise performance as a result of an increase in the stress response, where the activation of the hypothalamic-pituitary-thyroid axis (HPA) results in an increase in cortisol being secreted (Wright, Selkirk and McLellan, 2010; Daanen, Racinais and Périard, 2018; Reeve *et al.*, 2019). Within the current literature, an isothermic HA approach has been investigated either with an absolute increase in core temperature (Regan, Macfarlane and Taylor, 1996; Magalhães *et al.*, 2010) or the attainment of a set thermal strain (Garrett *et al.*, 2012; Gibson *et al.*, 2015). A potential limitation to using a set increase in core body temperature (e.g. +1 °C) is that as adaptation occurs and resting core temperature lowers, individuals may not be reaching a sufficient thermal strain to elicit HA adaptations. The attainment of a set thermal strain (e.g. 38.5 °C) ensures that as adaptation occurs an adaptation thermal stimulus continues to be provided (Fox *et al.*, 1964; Regan, Macfarlane and Taylor, 1996). Recent isothermic HA literature has shown that a daily 90 min isothermic HA protocol offers an adequate stimulus for thermal adaptations (Garrett *et al.*, 2012; Gibson *et al.*, 2015) but such a duration may be problematic to integrate in to athlete preparation. Shorter (30 min to 60 min) constant work approaches can induce beneficial thermal adaptations (Houmard *et al.*, 1990) when

exercise is maintained throughout to induce the strain and so it seems prudent to suggest that maintaining the strain for a similar duration using a less-intense exercise intensity, isothermic HA regimen would also be effective and desirable to tapering athletes. It is currently unknown whether such an approach provides enough time to induce physiological and perceptual adaptations as the time spent above the thermal impulse will be considerably reduced and total time exposed to the heat is ~33.3% less than previous isothermic HA protocols (Garrett *et al.*, 2012, 2014; Gibson *et al.*, 2015).

The primary aim of this study, therefore, was to investigate whether a 60 min daily isothermic (target rectal temperature of ~38.5 °C) HA regimen would reduce the physiological and perceptual strain experienced when exercising in the heat. The secondary aim was to investigate if there was a time-course effect on the physiological and perceptual adaptations and whether MTHA was more effective than STHA.

It was hypothesised that:

H_0: the isothermic HA protocol would not provide a sufficient thermal impulse to induce positive changes in physiological and perceptual measurements.

H_1: the isothermic HA protocol would provide a sufficient thermal impulse to induce positive changes in physiological and perceptual measurements.

H_2: adaptations to the physiological and perceptual systems would be more complete following MTHA than STHA.

5.2. Methods

5.2.1. Participants

Sixteen, non-heat acclimated, endurance runners (females = 3) (mean ± SD: age 36.1 ± 9.1 y, stature 176.1 ± 5.8 cm, body mass 74.2 ± 9.4 kg, body fat percentage 10.7 ± 4.9% and maximal workload (W_{max}) 302 ± 76 W) participated. Before participation, all participants completed a health screening questionnaire and provided their fully informed, written consent to participate. The study was approved by the University of Roehampton's ethical committee (LSC 18/228, Appendix C3) and all procedures and protocols adhered to the guidelines of the World Medical Association (*Declaration of Helsinki*). Data were collected between March and April (Mean outside temperature ~6 °C) in the United Kingdom to avoid heat acclimatisation. For calibration measurements of equipment used see Appendix E1.

5.2.2. Experimental design

Participants undertook one preliminary visit (for the assessment of maximal power output) and thirteen experimental visits. Participants performed a 45 min sub-maximal (40% W_{max}) heat stress test (HST) on the first (HST_{PRE}), seventh (HST_{STHA}) and thirteenth (HST_{MTHA})

experimental visit. Between HST_{PRE} and HST_{STHA} and again between HST_{STHA} and HST_{MTHA}, participants completed five consecutive days of isothermic heat acclimation (HA) (60 min each visit) during which time rectal temperature (T_{re}) was elevated to, and maintained at, ~38.5 °C. The environmental conditions were 40 °C and 50% relative humidity (rh) with no convective cooling for all sessions. Participants were instructed to avoid caffeine, alcohol, and strenuous exercise 24 h before all HSTs. HSTs and HA sessions were performed at the same time of day for each participant throughout the study to avoid the effects of circadian rhythm. Food intake was recorded for the 24 h prior to HST_{PRE} and participants were instructed to replicate this before HST_{STHA} and HST_{MTHA}. Environmental conditions, exercise intensity and length of exercise for HST were based upon both the current literature and pilot testing with non-cyclists. Individuals were unable to sustain an exercise intensity of 50% and 60% W_{max} for longer than 15 min, therefore a lower exercise intensity was chosen.

5.2.3. Preliminary testing

Stature (Harpenden Stadiometer, Holtain Ltd, UK) and body mass (Seca, Birmingham, UK) were recorded before W_{max} was determined in ambient laboratory conditions (21 ± 1 °C and 55 ± 4% rh) using an incremental exercise test to volitional exhaustion (Kuipers *et al.*, 1985), on a cycle ergometer (Monark 847E, Vansbro, Sweden). During this test, participants initially cycled at 100 watts (W) for 5 min, thereafter, work was increased

by 50 W every 2.5 min until heart rate (HR) reached 160 b.min^{-1}, once reached, work was increased by 25 W every 2.5 min until exhaustion. The maximum work rate was calculated using the equation of Kuipers *et al*. (1985): $W_{max} = W_{com} + ((t/150) \times \Delta W)$ [W_{com} = last work rate completed; t = duration (in seconds) of the final, uncompleted, stage; ΔW = final load increment (typically 21 W)] (Kuipers *et al.*, 1985). Percentage of body fat (%) was measured using whole body air displacement plethysmography method (BodPod, Cosmed, Italy).

5.2.4. Heat stress tests (HSTs)

Upon arrival, a mid-flow urine sample was provided to measure urine specific gravity (USG), using a hand-held pen refractometer (Atago, pen refractometer, PEN-Urine S.G, Tokyo, Japan). All participants reported to the laboratory euhydrated (USG < 1.020). Following this, participants self-recorded nude body mass (BM) and self-inserted a rectal thermistor (REC-U-VL3-0, Grant Instruments (Cambridge) Ltd., UK) ~10 cm past the anal sphincter before affixing a HR monitor (Polar Electro Ltd., Kempele, Finland) to their upper torso. The rectal thermistor was connected to a portable data logger (Squirrel 2020 Series, Grant Instruments, (Cambridge) Ltd., UK). Skin temperature was recorded continuously using wireless Thermochron iButton skin temperature data loggers (DS1922L, Thermochron iButton, USA). iButtons were attached using transparent adhesive dressing (Tegaderm, 3M Health Care, St Paul, MN) and waterproof tape (Transpore, 3M Health Care, St Paul, MN) to the sternal

notch (T_{sn}), forearm (T_f), thigh (T_t) & calf muscle (T_c) on the right side of the body. Mean-weighted skin temperature (T_{sk}) was calculated using the equation of Ramanathan ($T_{sk} = ([0.3 \times T_{sn}] + [0.3 \times T_f]) + [0.2 \times T_t] + [0.2 \times T_c]$) (Ramanathan, 1964) and mean body temperature (T_{body}) was estimated using the equation of Stolwijk and Hardy ($T_{body} = (0.79 \times T_{re}) + (0.21 \times T_{sk})$) (Stolwijk and Hardy, 1966). Due to methodological issues measuring T_{sk} in some participants, T_{body} data were collected from only seven participants.

Participants entered the controlled environment (40 °C, 50% rh; Weiss, Technik, UK) and rested for 2 min before baseline measurements of HR, T_{re}, thermal sensation (TS; Young *et al.*, 2002), and thermal comfort (TC; Gagge, Stolwijk and Hardy, 1967) were recorded and rated. Once baseline measurements were taken participants cycled at 40% of their W_{max} for 45 min, during which HR, T_{re}, ratings of perceived exertion (RPE; Borg, 1982), TS and TC were measured every 5 min. One min expired air samples were collected at 14 min, 29 min and 44 min using the Douglas bag method and subsequently analysed (1400 series, Servomex, East Sussex, UK; Harvard Dry Gas Meter, Harvard Ltd., Kent, UK). In order to prevent further, uncontrolled per-cooling, participants drank warm (~37 °C) water ad libitum. The water was stored in the environmental chamber and the volume consumed was recorded. Once final measurements were recorded, participants exited the controlled environment and self-recorded a final nude BM measurement after they had towel dried. Sweat losses

were determined from trial changes in BM, subtracting the weight of urine produced and adding fluid consumed (mL) during the trial.

5.2.5. Heat acclimation (HA)

Participants initially repeated the same procedures as undertaken in HST_{PRE}. After USG and BM were measured and a rectal thermistor was self-inserted, iButtons were placed on the same four sites and a HR strap was fitted to their upper torso before entering the controlled environment. Once baseline T_{re}, HR, TS, and TC measurements were recorded after 2 min rest, participants were instructed to reach a target T_{re} of ~38.5 °C as quickly as possible and self-selected their workload and cadence accordingly. Once the target T_{re} had been attained, the distance cycled, and the time taken to reach the target T_{re} were recorded, as was the time spent at or above it. Participants then sat for the remainder of the 60 min session unless T_{re} fell to 38.55 °C, at which point participants resumed cycling to increase T_{re}. During the 60 min, HR, T_{re}, RPE, TS, and TC were recorded every 5 min and on completion of the session nude BM was recorded to estimate sweat losses. Participants drank warm water ad libitum to prevent uncontrolled per-cooling (~37 °C). The water was stored in the environmental chamber and the volume consumed was recorded. Due to methodological issues T_{sk} data, and as a result T_{body} data were collected from only six participants. The peak intra-session strain was calculated as the peak T_{re} minus the starting T_{re} during each HA sessions. Peak

cumulative strain was then calculated as the total strain for the five (STHA) and ten day (MTHA) HA regimens.

5.2.6. Salivary cortisol sample collection and analyses

Saliva samples were collected from each participant immediately upon awakening on two of the five days before HST_{PRE} (B1 and B2) to establish normal basal concentrations and then again immediately before and after each HST. Saliva was collected by each participant chewing an absorbent swab (Salivette Cortisol, Code Blue, Sarstedt, Leicester, UK) then inserting it into a Salivette tube. All samples were centrifuged at 1,000 *g* for 2 min with the resulting saliva sample transferred into 2 mL Eppendorf tubes and stored in a freezer at -80 °C until analysis. Salivary cortisol levels were determined with a high sensitivity (0.007 $\mu g \cdot dL^{-1}$) salivary cortisol enzyme-linked immunosorbent assay (Salumetrics, State College, PA, USA) as per the manufacturer's instructions. The inter-assay variability (5.7%) was obtained from a high and low control sample in every assay.

5.2.7. Statistical analyses

Data were analysed using SPSS (version 26, SPSS Inc.). Parametric assumptions were met unless stated otherwise. One-way and two-way repeated measures ANOVAs were performed to determine differences between time points and trials in HST_{PRE}, HST_{STHA}, and HST_{MTHA}, and in the first, middle, and final HA session (HA1, HA5 & HA10). Where the

assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimate. Where significant outcomes were present, post-hoc tests with Bonferroni corrections were performed. The alpha level was $P < 0.05$. Cohen's *d* effect sizes were calculated for post-preload data and interpreted as follows: small effect: $d = 0.2$ to < 0.5; medium effect: 0.5 to < 0.8; large effect: $d \geq 0.8$ (Cohen, 1988). Data are presented as mean ± SD.

5.3. Results

5.3.1. Heat acclimation – (Table 5.1)

There was a main effect of trial for distance ($P = 0.025$) but not duration ($P = 0.108$) cycled before the attainment of the target T_{re} (38.5 °C). The distance was similar between HA1 (18 ± 4 km) and HA5 (21 ± 4 km; $P = 0.240$) but greater in HA10 (23 ± 5 km) compared to HA1 ($P = 0.018$) and HA5 ($P = 0.026$). It took longer to reach the target T_{re} in HA10 (41.6 ± 6.4 min) than in HA1 (35.7 ± 6.1 min) but this was not statistically significant. The duration was also similar between HA1 and HA5 (36.9 ± 6.8 min) and between HA5 and HA10. Despite differences in the distance cycled, the thermal impulse to 38.5 °C was not different between HA1 (0.032 ± 0.006 °C.min^{-1}), HA5 (0.033 ± 0.006 °C.min^{-1}), or HA10 (0.036 ± 0.007 °C.min^{-1}) (Main effect for trial: $P = 0.200$). The peak cumulative thermal strain and impulse provided by the STHA and MTHA interventions were 9.85 ± 1.35 °C and 0.033 ± 0.0042 °C.min^{-1} and 20.52 ± 2.26 °C and 0.034 ± 0.004 °C.min^{-1}, respectively. There were no changes in the classic

physiological or perceptual markers of heat adaptation measured before or during HA1, HA5, and HA10 (Table 5.1).

Table 5.1. Physiological and perceptual data on the first (HA1), fifth (HA5) and tenth (HA10) heat acclimation sessions. Data are presented as mean ± SD. (*Significant ($P < 0.05$) difference from HA1; #Significant ($P < 0.05$) difference (from HA5).

		HA1	HA5	HA10
Temperature	Resting T_{re} (°C)	36.92 ± 0.36	36.83 ± 0.34	36.71 ± 0.36
	End T_{re} (°C)	38.84 ± 0.27	38.80 ± 0.25	38.85 ± 0.30
	Mean T_{sk} (°C)	36.37 ± 0.33	36.37 ± 0.50	35.70 ± 0.58
	T_{body} (°C)	37.58 ± 0.83	37.59 ± 0.88	37.41 ± 1.05
	Total ROR ($°C \cdot h^{-1}$)	1.92 ± 0.34	1.97 ± 0.36	2.14 ± 0.44
Heart Rate	Resting HR ($b.min^{-1}$)	75 ± 16	73 ± 13	72 ± 15
	Mean HR ($b.min^{-1}$)	135 ± 13	133 ±13	136 ± 9
Sweat Loss/Fluid	Sweat loss ($L \cdot h^{-1}$)	1.39 ± 0.41	1.75 ± 0.81	1.76 ± 0.56
	Fluid consumption (L)	0.79 ± 0.20	1.01 ± 0.47	1.05 ± 0.49
Perceptual Measurements	Resting TC	1.1 ± 0.3	1.3 ± 0.4	1.1 ± 0.3
	Mean TC	2.2 ± 0.5	2.0 ± 0.4	1.9 ± 0.4
	Resting TS	4.6 ± 0.6	4.3 ± 0.9	4.3 ± 0.7
	Mean TS	5.7 ± 0.6	5.6 ± 0.6	5.5 ± 0.5
	Mean RPE	13 ± 2	13 ± 2	13 ± 2

N=16 for all data except for skin and mean body temperature (n=6). Mean data are for the 60 min session except for RPE which are the mean of the time spent exercising only.

5.3.2. Heat stress test – Physiological data (Table 5.2)

There was a main effect for trial on resting T_{re} ($P < 0.001$) and T_{body} ($P < 0.001$) with both lower in HST_{STHA} ($P < 0.001$, $d = 1.2$; $P < 0.006$, $d = 1.6$) and HST_{MTHA} (both $P < 0.001$, $d = 1.3$ and 1.6 respectively) than HST_{PRE}, whereas no difference in resting T_{sk} was measured between HSTs ($P = 0.243$, $d = 0.3 – 0.7$). Resting HR was similar between HST_{PRE} and both HST_{STHA} ($P = 0.244$, $d = 0.6$) and HST_{MTHA} ($P = 0.113$, $d = 0.7$) despite a significant main effect ($P = 0.042$). There were no differences in resting T_{re}, T_{body}, or HR between HST_{STHA} and HST_{MTHA} (all $P > 0.99$, $d < 0.2$).

The cardiovascular and thermoregulatory strain experienced was lower during (Main effects: $P < 0.007$) and at the end (Main effects: $P < 0.005$) of the HSTs performed following HA (Figure 5.1 and Figure 5.2). Mean T_{re}, T_{sk} and T_{body} were higher during HST_{PRE} compared to HST_{STHA} ($P = 0.002$, $d = 1.1$; $P = 0.026$, $d = 1.3$; $P = 0.007$, $d = 1.5$, respectively) and HST_{MTHA} ($P < 0.001$, $d = 1.4$; $P = 0.034$, $d = 1.3$; $P = 0.005$, $d = 1.5$, respectively) but were similar in HST_{STHA} and HST_{MTHA} ($P = 0.223$, $d = 0.6$; $P > 0.99$, $d = 0.1$; $P = 0.692$, $d = 0.5$, respectively). The rise in T_{re} over the exercise bout was similar in all three HSTs ($P = 0.292$). T_{re} was lower at all time-points in HST_{MTHA} compared to HST_{PRE} ($P < 0.05$) and for the first 40 min in HST_{STHA} compared to HST_{PRE} (all $P < 0.05$). T_{re} was similar in HST_{STHA} and HST_{MTHA} at each time point (all $P > 0.05$) (Figure 5.2). Mean HR was higher in HST_{PRE} than HST_{STHA} ($P = 0.004$, $d = 0.8$) and HST_{MTHA} ($P = 0.004$, $d = 1.1$) but was similar between HST_{STHA} and

HST_{MTHA} (P = 0.074, *d* = 0.6). HR was higher in HST_{STHA} than HST_{MTHA} at 10 (P = 0.034), 15 (P = 0.018), and 20 min (P = 0.035) during the HST (Figure 5.1). After 45 min of exercise, T_{re} was not different between HST_{PRE} compared to HST_{STHA} (P = 0.081, *d* = 0.7) but was lower in HST_{MTHA} compared to HST_{PRE} (P < 0.001, *d* = 1.1), there were no differences between HST_{STHA} and HST_{MTHA} (P = 0.488, *d* = 0.4). T_{sk} and T_{body} was lower in both HST_{STHA} (P = 0.044, *d* = 1.2; P = 0.048, *d* = 1.3) and HST_{MTHA} (P = 0.018, *d* = 1.0; P = 0.008, *d* = 1.6) compared to HST_{PRE} but no differences were seen between HST_{STHA} and HST_{MTHA} (all P > 0.99, *d* = 0.41; *d* = 0.27). Final HR was higher in HST_{PRE} than HST_{STHA} (P = 0.012, *d* = 0.6) and HST_{MTHA} (P = 0.003, *d* = 1.0) but no differences were observed between HST_{STHA} and HST_{MTHA} (P = 0.065, *d* = 0.7). Sweat rate was similar in HST_{PRE} and HST_{STHA} (P > 0.99), but higher during HST_{MTHA} than HST_{PRE} (P < 0.001, *d* = 1.0) and HST_{STHA} (P < 0.001, *d* = 0.8). Fluid consumption was similar in all trials (main effect: P = 0.827) but the percentage dehydration differed (main effect: P < 0.001), being greater in HST_{MTHA} compared to both HST_{STHA} (P < 0.001, *d* = 1.1) and HST_{PRE} (P < 0.001, *d* = 0.8). $\dot{V}O_2$ and respiratory exchange ratio (RER) were similar between trials (main effect trial: P = 0.094; P = 0.089) and did not change over time (P = 0.515, P = 0.116).

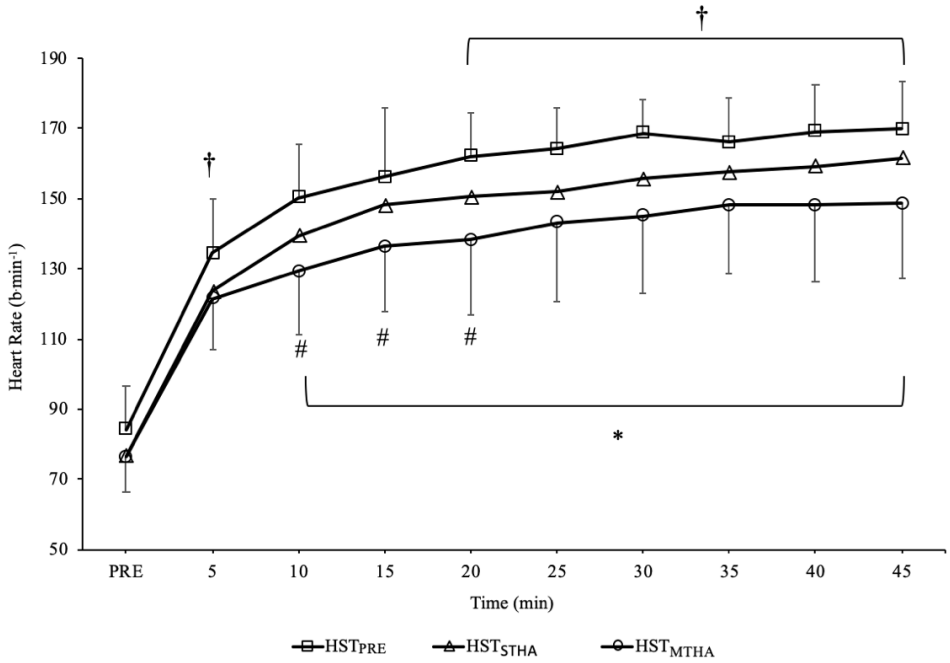

Figure 5.1. Heart rate during HST_{PRE}, HST_{STHA}, and HST_{MTHA}. †Significant ($P < 0.05$) difference between HST_{PRE} and HST_{STHA} *Significant ($P < 0.05$) difference between HST_{PRE} and HST_{MTHA}. #Significant ($P < 0.05$) difference between HST_{MTHA} and HST_{STHA}. Data mean ± SD (n = 16).

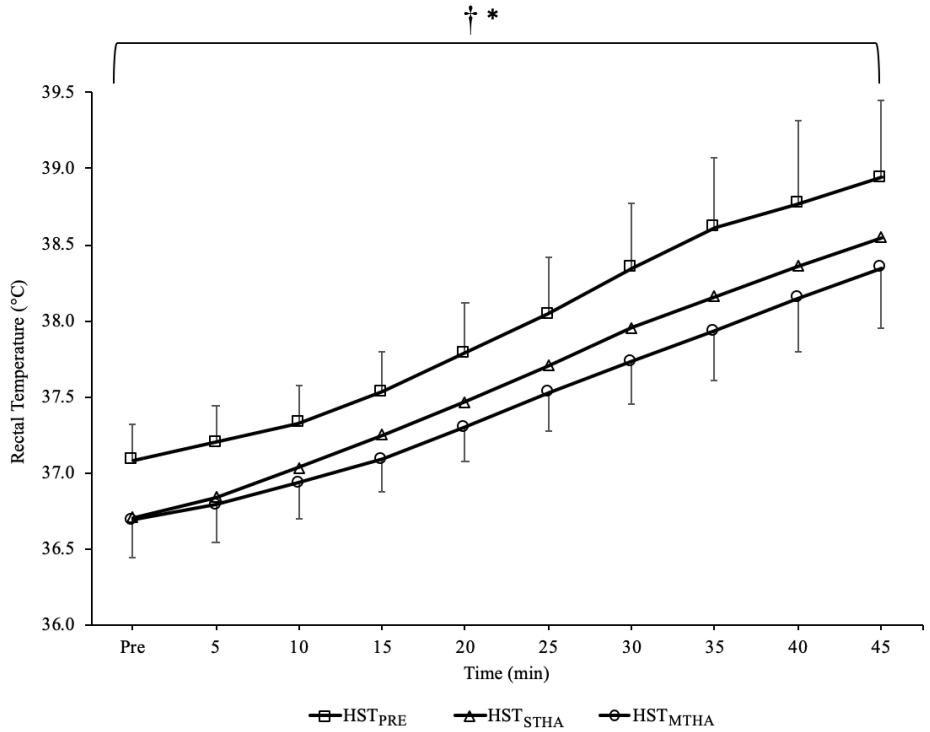

Figure 5.2. Rectal temperature (T_{re}) at each time point during HST_{PRE}, HST_{STHA}, and HST_{MTHA}. †Significant ($P < 0.05$) difference between HST_{PRE} and HST_{STHA} *Significant ($P < 0.05$) difference between HST_{PRE} and HST_{MTHA}. Data mean ± SD (n = 16).

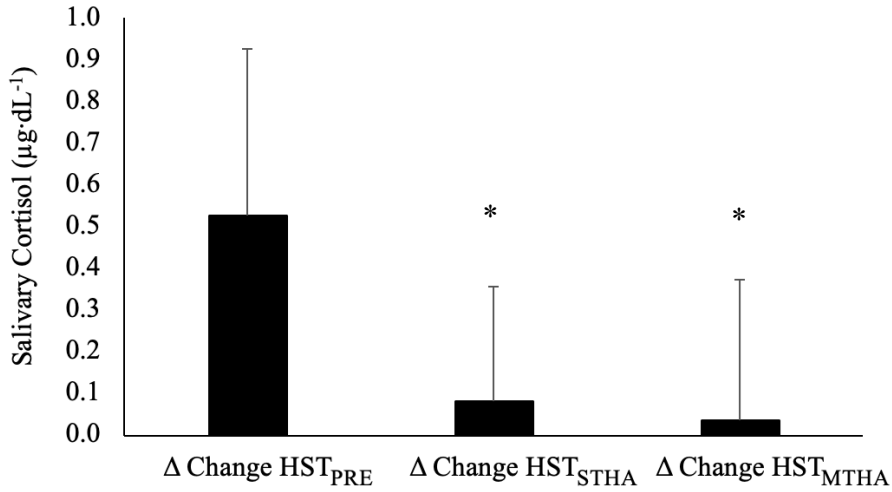

Figure 5.1. Mean ± SD delta (Δ) change (Pre - Post HST) in salivary cortisol within trials (n = 16). *Significant ($P < 0.05$) difference compared to HST_{PRE}.

Baseline cortisol concentrations and the concentrations prior to all HSTs were similar among the trials (all $P > 0.05$). The coefficient of variation between B1 and B2 was 15 ± 6%. The within trial increase was different between trials ($P < 0.001$) being greater in HST_{PRE} (0.53 ± 0.40 µg·dL^{-1}; +339 ± 284%) compared to HST_{STHA} (0.08 ± 0.29 µg·dL^{-1}, $P < 0.001$, d = 1.1; +90 ± 183%) and HST_{MTHA} (0.04 ± 0.35 µg·dL^{-1}, $P = 0.003$, d = 1.1; +93 ± 181%) (Figure 5.1). There were no differences in the within trial change in cortisol between HST_{STHA} and HST_{MTHA} ($P > 0.99$, d = 0.2). There was a large degree of variation in the percentage change within trials in HST_{PRE} (-37 - +730%), HST_{STHA} (-62 - 550%), and HST_{MTHA} (-63 - 580%); however, the number of participants who had an increase in

cortisol concentration in their post- sample compared to their pre- sample was higher in HST_{PRE} (10/12) than HST_{STHA} (6/12) and HST_{MTHA} (6/12).

5.3.3. Heat stress test – Perceptual measurements (Table 5.2)

There were main effects of trial and time for TS, TC, and RPE (all $P < 0.002$), with all increasing progressively throughout the HST (all $P < 0.001$), there was an interaction effect for TC and RPE (all $P < 0.006$) but not for TS ($P = 0.248$). Resting TC was unaffected by HA ($P = 0.487$) but resting TS was different between trials ($p < 0.001$) being lower in HST_{STHA} ($P = 0.014$) and HST_{MTHA} ($P = 0.002$). All of the reduction had occurred within five days of HA with no differences between HST_{STHA} and HST_{MTHA} ($P > 0.99$). During exercise, mean TS, TC, and RPE were different between trials ($P < 0.001$). TS and TC were both higher in HST_{PRE} than HST_{STHA} ($P < 0.001$, $P = 0.037$) and HST_{MTHA} ($P < 0.002$, $P < 0.001$). Both were further reduced from HST_{STHA} to HST_{MTHA} (TS; $P = 0.031$, TC; $P = 0.030$) (Figure 5.2). Mean RPE was not different between HST_{PRE} and HST_{STHA} ($P = 0.456$) but was lower in HST_{MTHA} compared to HST_{PRE} ($P = 0.006$) and HST_{STHA} ($P = 0.015$). At the end of exercise, TS, TC, and RPE were all lower after HST_{STHA} ($P < 0.001$, $P = 0.039$, $P = 0.037$) and HST_{MTHA} (all $P < 0.001$) compared to $HST_{PRE.}$ Thermal sensations were rated lower at the end of HST_{MTHA} than HST_{STHA} ($P = 0.046$; $d = 0.56$) but neither TC ($P = 0.083$; $d = 0.52$) nor RPE ($P = 0.120$; $d = 0.13$) were rated differently between HST_{STHA} and HST_{MTHA}. Data are reported in Table 5.2.

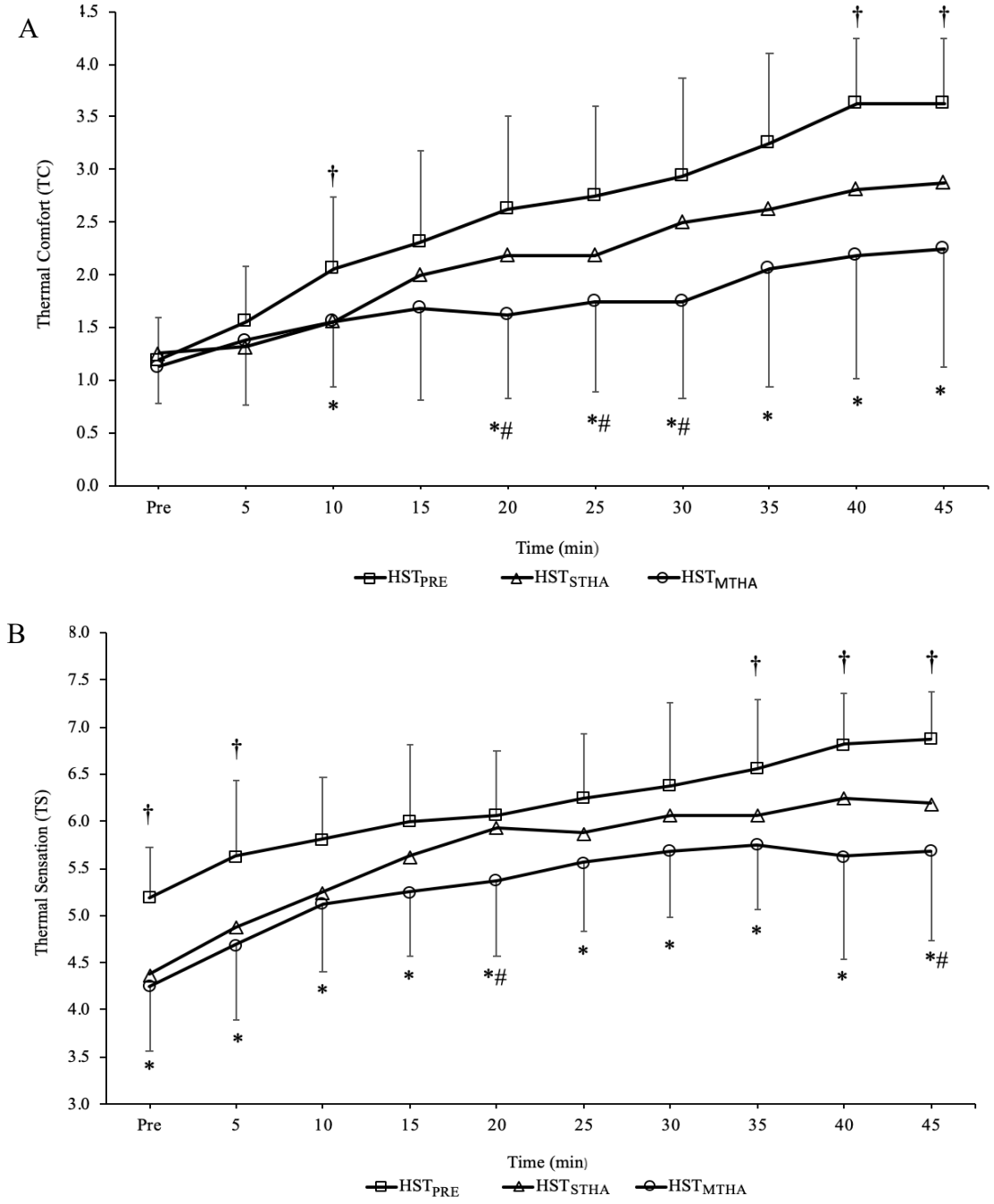

Figure 5.2. (A) Thermal comfort (TC) and (B) Thermal sensation (TS) were rated pre- and every 5 min during HSTs. †Significant ($P < 0.05$) difference between HST_{PRE} and HST_{STHA}. *Significant ($P < 0.05$) difference between HST_{PRE} and HST_{MTHA}. #Significant ($P < 0.05$) difference between HST_{MTHA} and HST_{STHA}. Data mean ± SD (n = 16).

Table 5.2. Physiological and perceptual responses to the heat stress tests (mean ± SD) (*Significant ($P < 0.05$) difference from HST_{PRE}; #Significant ($P < 0.05$) difference from HST_{STHA}).

		HST_{PRE}	HST_{STHA}	HST_{MTHA}
Resting	T_{re} (°C)	37.09 ± 0.23	36.70 ± 0.27*	36.69 ± 0.24*
	T_{sk} (°C)	35.52 ± 0.48	35.39 ± 0.38	34.84 ± 1.24
	T_{body} (°C)	36.76 ± 0.15	36.32 ± 0.14*	36.28 ± 0.18*
	HR ($b.min^{-1}$)	84 ± 12	77 ± 12	76 ± 10
	TC	1.3 ± 0.4	1.3 ± 0.4	1.1 ± 0.3
	TS	5.3 ± 0.4	4.4 ± 0.8*	4.3 ± 0.7*
Mean	T_{re} (°C)	37.97 ± 0.30	37.60 ± 0.28*	37.45 ± 0.21*
	T_{sk} (°C)	37.21 ± 0.1	36.68 ± 0.1*	36.87 ± 0.5*
	T_{body} (°C)	37.81 ± 0.74	37.25 ± 0.66*	37.34 ± 0.67*
	HR ($b.min^{-1}$)	153 ± 11	142 ± 12*	134 ± 17*
	$\dot{V}O_2$	2.2 ± 0.8	2.1 ± 0.7	1.7 ± 0.8
	RER	0.84 ± 0.1	0.84 ± 0.1	0.74 ± 0.1
	RPE	14 ± 0.6	13 ± 0.5	12 ± 0.5*#
	TC	2.6 ± 0.7	2.1 ± 0.9*	1.9 ± 0.8*#
	TS	6.2 ± 0.6	5.7 ± 0.8*	5.4 ± 0.7*#
End	T_{re} (°C)	38.94 ± 0.51	38.55 ± 0.57	38.35 ± 0.39*
	T_{sk} (°C)	38.11 ± 0.3	37.26 ± 0.5*	37.51 ± 0.5*

T_{body} (°C)	38.83 ± 0.42	38.08 ± 0.44*	38.19 ± 0.35*
HR ($b.min^{-1}$)	170 ± 13	162 ± 13*	149 ± 21*
RPE	16 ± 3	14 ± 3*	12 ± 3*
TC	3.7 ± 0.6	2.9 ± 1.3*	2.3 ± 1.1*
TS	6.8 ± 0.5	6.2 ± 0.8*	5.8 ± 0.9*#
Change T_{re} (°C)	1.85 ± 0.57	1.84 ± 0.65	1.66 ± 0.50
Sweat Rate ($L{\cdot}h^{-1}$)	0.94 ± 0.15	1.06 ± 0.23	1.40 ± 0.30*#
Fluid Consumed ($L{\cdot}h^{-1}$)	0.74 ± 0.15	0.70 ± 0.17	0.71 ± 0.25
Dehydration (%)	-1.70 ± 0.30	-1.79 ± 0.43	-2.15 ± 0.39*#

N = 16 for all data except for skin (T_{sk}) and mean body temperature (T_{body}) (n = 7).

5.4. Discussion

The present study investigated whether a daily 60 min isothermic HA protocol provided a sufficient thermal impulse to induce the physiological and perceptual adaptations and whether there was a time course response when comparing STHA and MTHA. The main findings of the present study are: (1) an isothermic STHA protocol provides a sufficient cumulative thermal strain (9.85 ± 1.35 °C) to effectively lower physiological and perceptual strain and (2) MTHA induces further beneficial heat adaptations but only to sweat losses, final thermoregulatory strain, perceptions of thermal strain and comfort, despite providing double the cumulative thermal strain (20.52 ± 2.26 °C).

5.4.1. Physiological adaptations

The isothermic HA protocol was successful at lowering resting T_{re} (-0.38 ± 0.26 °C) and HR (8 ± 16 b.min^{-1}) after five days of heat exposure. A further five days of heat acclimation (MTHA) did not elicit any further resting adaptations in T_{re} (-0.02 ± 0.31 °C from STHA) or HR (0 ± 9 b.min^{-1} from STHA). Both these responses are in accordance with previous findings that reported that these adaptations occurred at a rapid rate (Nielsen *et al.*, 1993) and were not further enhanced after longer exposure periods (Tyler *et al.*, 2016). A lower resting T_{re} is an important indicator of a successful HA protocol because it can delay the attainment of high core body temperatures often reported to limit exercise capacity in the heat

(Nielsen *et al.*, 1993; González-Alonso *et al.*, 1999). Our observed reductions are greater than mean changes reported in a recent meta-analysis (STHA: -0.17 ± 0.12 °C; MTHA: -0.17 ± 0.1 °C) (Tyler *et al.*, 2016) and those reported in previous five day isothermic-controlled studies (Garrett *et al.*, 2012, 2014; Neal *et al.*, 2016). The more substantial reductions in physiological markers of heat acclimation may be due to the lower training status of our participants compared to those presented in the literature, where comparably smaller reductions in resting T_{re} were observed in more highly trained (mean peak power output of 375 ± 31 W) individuals (Garrett *et al.*, 2012, 2014; Neal *et al.*, 2016). This is likely because highly trained individuals have already developed some thermal adaptations from their long-term training history (e.g. a greater evaporative heat loss capacity and a decrease in resting core temperature) (Cheung and McLellan, 1998a) which would limit the potential for a HA protocol to induce further adaptations.

The reduction in resting T_{re} coupled with similar (HST_{PRE}: 1.85 ± 0.57 °C, HST_{STHA}: 1.84 ± 0.65 °C) and lower (1.66 ± 0.50 °C) delta changes in T_{re} after five and ten days of HA, respectively resulted in reduced thermal strain throughout HST_{STHA} and HST_{MTHA} compared to HST_{PRE}. HA has been shown to reduce the oxygen cost at a given intensity in the heat (Lorenzo *et al.*, 2010) and enhance lipid oxidation (Kirwan *et al.*, 1987); however, this is not always reported when using cycling as the mode of exercise, where the utilisation of the upper body muscles are minimal. This

might explain why $\dot{V}O_2$ and RER were not altered following either STHA or MTHA in the present study. Due to the lack of change in efficiency, it seems reasonable to assume that the reduced thermal strain was due to an increase in heat loss mechanisms, as sweat rate increased after ten days of HA, facilitating a greater heat loss through evaporative cooling. Our data support previous findings, that found sudomotor responses took longer to occur than other adaptations (Nielsen *et al.*, 1993; Cheung and McLellan, 1998b; Lee *et al.*, 2016; Tyler *et al.*, 2016). While local methods to access sweat rates were not used in the current study, whole body sweat rates were increased following ten (MTHA) but not five (STHA) days of heat exposure. Previous results have found that HA increases sweat rate, as a result of an earlier onset of sweating at a lower core temperature and a more pronounced sudomotor thermosensitivity (Buono *et al.*, 2018).

It is well known that cardiovascular strain can limit prolonged exercise performed under heat stress (Lee and Scott, 1916; Périard *et al.*, 2011) and reduced cardiovascular strain is a classic marker of an effective HA regimen. In the present study, resting HR was unaffected by HA; however, the mean exercising, and end of exercise HR was reduced following STHA. There was no additional benefit of a longer exposure period (MTHA) on resting and end of exercise HR; however, longer exposure time lowered HR response at certain time points during exercise. Adaptations in HR occur rapidly and are often complete within seven days (Périard, Racinais and Sawka, 2015) and data from the present study

support this. While physiological measurements such as stroke volume, skin blood flow and plasma volume were not measured in the current study, identifying the factor that influenced this response is can only be speculated. It has been previously suggested that the improved cardiovascular stability from HA, is achieved through an increase in plasma volume, a better maintained fluid balance, and enhanced sweating and skin blood flow responses (Périard, Racinais and Sawka, 2015; Périard *et al.*, 2016).

Cortisol is often used as a marker of physical and psychological strain and, as observed elsewhere (e.g. Silva *et al.*, 2019), cortisol concentrations increased following an initial bout of exercise in the heat (HST_{PRE}). Following HA we observed an attenuated increase – data which are in contrast to Costello *et al*. (2018) and Garrett *et al.* (2009) but in agreement with Watkins *et al.* (2008) who reported reductions in the session increase in cortisol after seven days of HA. Costello *et al.* (2018) did not report statistical reductions in cortisol following HA but noted that there was a "trend" for the increase to be lower post-HA and so the cortisol response to exercise in the heat may be sensitive to heat adaptation. While cortisol may be a potential marker of heat adaptation, due to the variation within and between investigations it is advisable to use the more established variables (e.g. resting core body temperature and heart rate) at present.

5.4.2. Perceptual adaptations

Participants felt more thermally comfortable and reported lower thermal sensations after five days of HA (STHA). An extra five days of HA (MTHA) had an additional beneficial effect on final thermal sensations. Both T_{re} and T_{sk} are key drivers of thermal perceptions, but in the present study, neither T_{re} nor T_{sk} continued to decline with longer exposure and so these observations do not explain why these perceptual responses continued to be improved during the HA regimen. An increase in perceived exertion (RPE) and thermal perceptions (TS, TC), have been reported to play a role in downregulating self-paced time trials (TT) when performed under heat stress in order to reduce the rate of heat storage well before hyperthermia is present (Tucker *et al.*, 2004). Although not measured, it seems reasonable to suggest that a lower perceived exertion and improved perceptions of thermal strain and comfort would enable participants to select a higher exercise intensity and improve subsequent performance as reported elsewhere (Tyler, Wild and Sunderland, 2010; Burdon *et al.*, 2013). Our data suggest that the five day isothermic STHA regimen provided a sufficient thermal stimulus to improve perceptions of strain but MTHA (ten day) offered further benefit and so is the preferred approach.

5.4.3. Limitations and practical recommendations

We cannot exclude the possibility that there was a training effect that may have occurred during HA as there was no passive control group; however,

in previous studies that included a control group, there was no reported training benefit in performance outcomes (Lorenzo *et al.*, 2010). Additionally, the intensity and duration of exercise used in the present study was substantially lower than the participants were used to, as all participants were about to take part in the Marathon des Sables, a 250 km foot-race across the Sahara Desert.

Identifying an effective heat acclimation protocol that reduces the risk of overexerting an athlete so close to competition, while optimising thermal adaptations, is of current focus while athletes prepare for upcoming sporting events, including the Olympic Games in Tokyo, 2020. We did not measure whether the isothermic STHA and MTHA regimens improved subsequent exercise performance or reduced heat illness risk but we speculate that progressive improvements would have been observed as a result of the reductions in physiological and perceptual strain as has been reported previously (Lorenzo *et al.*, 2010). We suggest using an isothermal HA regimen during the taper phase of an athlete's schedule and highlight that although five days is sufficient to induce meaningful beneficial adaptations to heat, ten days is more effective and so should be used when possible.

5.5. Conclusion

A five day (STHA) 60 min daily isothermic HA regimen (target rectal temperature ~38.5 ºC) provides a sufficient thermal stimulus to elicit

beneficial adaptations to reduce physiological and perceptual strain during subsequent exercise in the heat despite providing a lower cumulative thermal strain than commonly observed in the HA literature. Most of the beneficial adaptations occurred within the STHA time-frame; however, an additional five days of HA (MTHA) induced further cardiovascular, sudomotor, and perceptual adaptations and so isothermic MTHA is preferred over isothermic STHA when possible. The results of the present study reject H_0 and accepts both H_1 and H_2, however yet to be determined was whether an isothermic heat acclimation protocol could offer protective benefits to cognition function without inducing a leakage of lipopolysaccharide (LPS) into the systemic circulation. This is addressed in the upcoming experimental chapters.

Chapter 6. **The Effects of a Short-term and Medium-term Isothermic Heat Acclimation Protocol on Cognitive Function**

6.1. Introduction

It is well-documented that environmental heat stress can impair aerobic exercise capacity and performance (Galloway and Maughan, 1997; Tatterson *et al.*, 2000; Tucker *et al.*, 2004) but the impact on cognitive function is less clear. Cognitive function, in this context, defines performance within tasks that require conscious mental effort (Lamport *et al.*, 2014), and performance in such tasks may be compromised by a rise in brain, core and/or skin temperature and the complexity of the cognitive task (Gaoua *et al.*, 2011; Taylor *et al.*, 2016). Impaired cognitive function is associated with an increase in mental fatigue, which is highly influential on exercise tolerance and the perception of effort and can be detrimental to endurance exercise performance (Blackwood *et al.*, 1998; Marcora, Staiano and Manning, 2009).

The data regarding the effects of thermal strain on cognitive function are equivocal. For example, high levels of thermal strain have been shown to have adverse effects on some complex cognitive tasks (i.e. working memory, information retention & processing) (Racinais, Gaoua and Grantham, 2008; Gaoua *et al.*, 2012) but a more mild thermal strain has been shown to improve certain simple cognitive functions (e.g. reaction time) (Nunneley and Maldonado, 1983; Simmons *et al.*, 2008; Gaoua *et al.*, 2011). This uncertainty exists due to a number of methodological inconsistencies between studies including (and not limited to) the duration of heat exposure, exercise tasks, change in body temperature, the cognitive tasks

employed, participants characteristics, and hydration status (Hancock & Vasmatzidis, 2003; Taylor *et al.*, 2016). Despite the mixed data and methodological differences, complex cognitive tasks appear to be impaired above a core temperature threshold of ~38.5 ºC and even simple tasks are impaired at very high levels of thermal strain (~40 ºC) (Gaoua *et al.*, 2018). Given the augmented physiological and perceptual strain associated with exhaustive exercise in the heat, it is reasonable to suggest that under these physiological stressful conditions, cognitive function is for the most part impaired (Nybo and Nielsen, 2001; Otani *et al.*, 2017).

A salient question, therefore, is can interventions such as heat acclimation (HA), that reduce the physiological strain associated with exercise heat stress be used to offset the reduction in cognitive function. HA may offer protection against the decline in cognitive function induced by thermal stress by lowering thermoregulatory strain and increasing heat exchange mechanisms. There is a lack of research directly investigating this question but there is some evidence to show that acclimatised individuals living in warm locations preserve cognitive function better in the heat compared to those who are not habitually heat acclimated (Wijayanto *et al.*, 2017). Of the limited data available, a 14 day and 28 day HA protocol was not found to protect against the detrimental effects of heat stress on complex motor tasks (Piil *et al.*, 2019). In this study, the HA protocol used was a fixed intensity exercise bout (60% $\dot{V}O_{2max}$) in trained men and a potential limitation to using a fixed intensity HA protocol is that as adaptation

occurs, individuals may not be attaining a sufficient thermal strain (relative to the start of HA) to induce adaptation. With the magnitude of adaptation appearing to be dependent on the impulse of thermal strain, it may be possible that these studies did not induce a thermal challenge of a magnitude above an adaptation threshold (Taylor and Cotter, 2006; Gibson *et al.*, 2015; Tyler *et al.*, 2016). For thermal adaptations to be induced it has been proposed that the attainment and maintenance of a core temperature of ~38.5 °C is necessary (Gibson *et al.*, 2015) because at this temperature sudomotor and vasomotor thermoeffector responses are challenged (Fox *et al.*, 1964). Gibson *et al.* (2015) reported that a 90 min isothermic HA protocol (target rectal temperature ~38.5 °C) was effective at inducing the necessary adaptations to reduce physiological and perceptual strain during exercise heat stress; however, this duration may lack practical application. Shorter (30 min to 60 min) constant work approaches can induce beneficial heat adaptations (Houmard *et al.*, 1990) when exercise is maintained throughout to induce the strain. Therefore, it seems prudent to suggest that an isothermic HA protocol, that maintains thermal strain for a similar duration using a less-intense exercise intensity would also be effective and desirable to tapering athletes.

Therefore, the primary aim of the present study was to investigate whether an acute bout of exercise is detrimental to cognitive function when measured from pre- to post-exercise under heat stress and whether a daily 60 min isothermic (target rectal temperature of ~38.5 °C) HA protocol

would offset any impairments in cognitive function from acute heat stress on in moderately-trained endurance individuals. The secondary aim was to determine if there was a time-course protective effect on cognitive function, and whether ten days (medium-term heat acclimation (MTHA)) was more effective than five days (short-term heat acclimation (STHA)).

It was hypothesised that:

H_{01}: an acute bout of exercise under heat stress would not impair cognitive function when measured from pre- to post-exercise.

H_1: an acute bout of exercise under heat stress would impair cognitive function when measured from pre- to post-exercise.

H_{02}: an isothermic HA protocol has no effect on offsetting the detrimental effects of exercise heat stress on cognitive function.

H_2: an isothermic HA protocol would offset the detrimental effects of exercise heat stress on cognitive function.

H_3: this outcome would be greater following MTHA than STHA.

6.2. Methods

6.2.1. Participants

The data presented in this experimental chapter were collected during the data collection of Chapter 5. Twelve (female = 2) out of the sixteen, non-heat acclimated, endurance-trained runners (mean ± SD: age 32.8 ± 13.7 y, stature 176.7 ± 6.2 cm, body mass 76.6 ± 9.2 kg, body fat percentage 10.7 ± 4.9% and maximal workload (W_{max}) 273 ± 122.6 W) participated in the present study, due to time-restrictions and equipment availability. Before participation, all participants completed a health screening questionnaire and provided written fully informed consent to participate. The study was approved by the University of Roehampton's ethical committee (LSC 18/228, Appendix C3) and all procedures and protocols adhered to the guidelines of the World Medical Association (*Declaration of Helsinki*). Data were collected between March and April (mean outside temperature ~6 °C) in the United Kingdom to avoid heat acclimatisation. For calibration of the equipment used see Appendix E1.

6.2.2. Experimental procedures

Full experimental details are presented in Chapter 5. In brief, participants undertook one preliminary visit (for assessment of maximal power output) and 13 consecutive experimental visits. Participants performed a 45 min sub-maximal (40% W_{max}) heat stress test (HST) on the first (HST_{PRE}), seventh (HST_{STHA}) and thirteenth (HST_{MTHA}) experimental visit. Between

HST_{PRE} and HST_{STHA} and again between HST_{STHA} and HST_{MTHA}, participants completed five consecutive days of an isothermic heat acclimation (HA) (60 min each visit) during which time rectal temperature (T_{re}) was elevated to, and maintained at, ~38.5 °C. The environmental conditions were 40 °C and 50% relative humidity (rh) with no convective cooling applied for all sessions. A battery of cognitive function tests were performed pre- and post-HST_{PRE}, and repeated again during HST_{STHA} and HST_{MTHA} (Figure 6.1). Participants were instructed to avoid caffeine, alcohol, and strenuous exercise 24 h before all HSTs. HSTs were performed at the same time of day for each participant throughout the study to avoid the effects of circadian rhythm. Food intake was recorded for the 24 h prior to HST_{PRE} and participants were instructed to replicate this before HST_{STHA} and HST_{MTHA}.

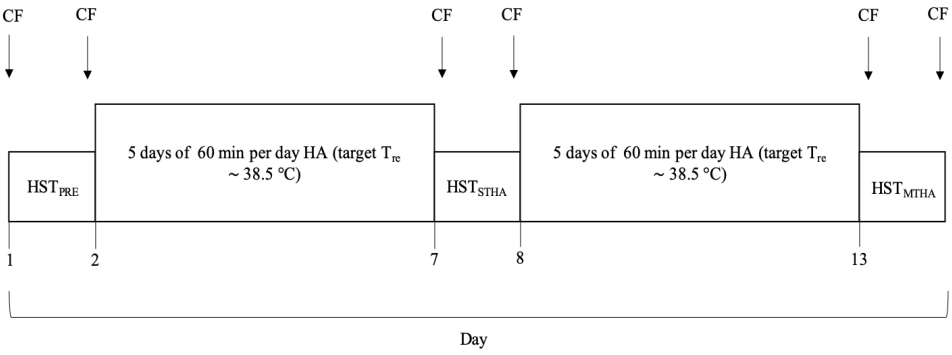

Figure 6.1. Schematic layout of study design. Participants completed a battery of cognitive function (CF) test pre- and post- each heat stress test (HST) on the first (HST_{PRE}), seventh (HST_{STHA}) & thirteenth (HST_{MTHA}) visit. Full details of experimental protocol are presented in Chapter 5.

6.2.3. Experimental trials – Heat stress test (HST)

Upon arrival participants provided a mid-flow urine sample to measure urine specific gravity (USG) before participants self-recorded their nude body mass, and then self-inserted a rectal thermistor. iButtons were attached to the four locations to continuously measure skin temperature and a heart rate (HR) monitor was affixed to the upper torso before participants entered the controlled environmental chamber (Weiss, Technik, UK). Following a resting period (2 min) baseline measurements for rectal temperature (T_{re}), HR, thermal comfort (TC) (Gagge, Stolwijk and Hardy, 1967) and sensation (TS) (Young *et al.*, 1987) were recorded.

6.2.4. Cognitive function

Participants completed a battery of cognitive function tests, for the assessment of choice reaction time and spatial working memory. The test lasted ~11 min and was completed before and after a 45 min sub-maximal exercise bout. All tests were conducted on an iPad® (Apple, USA) using the Cambridge Neuropsychological Test Automated Battery software (CANTAB, Cambridge Cognition, UK). These tests were chosen based on a previous experimental study conducted within our laboratory that used this software and therefore, the researcher was familiar with the testing method used. During the experimental testing, only two participants were in the environmental chamber (40 ºC, 50% rh) at the same time, where they were separated by a curtain and wore headphones to minimise interference.

6.2.4.1. Choice reaction time (RT)

In this test, the participant had to respond to a stimulus presented (a yellow light/flash) which appeared in one of five randomly allocated locations on the screen (Figure 6.2). The two measurements of RT were recorded as mean values in milliseconds (ms);

1. Response time: The mean time taken to release the button (blue) that was held at the start of each test once the stimulus had flashed.
2. Movement time: The mean time taken for the participant to touch the stimulus after the button had been released.

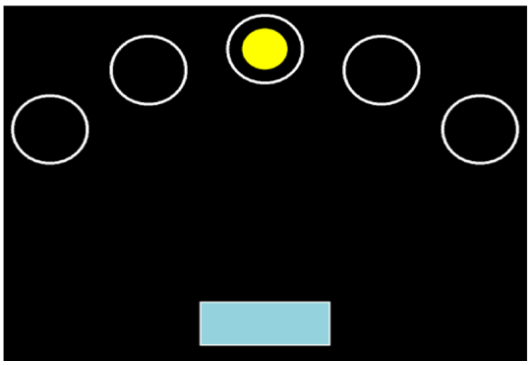

Figure 6.2. Reaction time response recorded. 1) Response time and 2) Movement time.

6.2.4.2. Spatial working memory

In this test, participants were presented with a number of coloured boxes beginning with four at the start and increasing to twelve by the end of the test. This test required participants to retain the visuospatial information and, through the process of elimination, participants were required to select boxes in search of the yellow token. Once found, the token was

placed on the right-hand side of the screen (Figure 6.3). Outcome measure for spatial working memory were;

1. The number of times a box was revisited in which a token had already been found.

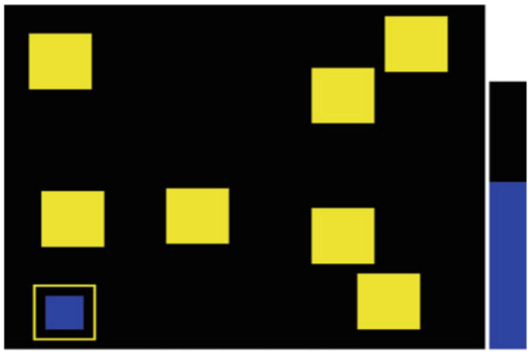

Figure 6.3. Spatial working memory measured the mean amount of errors during each task complexity - 4, 6, 8 and 12 boxes.

6.2.5. Statistical analyses

Data were analysed using SPSS (version 26, SPSS Inc.). Parametric assumptions were met unless stated otherwise. A one-way ANOVA was used to measured differences between trials pre-exercise and post-exercise and a repeated measures ANOVA were performed to determine differences in time points and trials in HSTs (HST_{PRE}, HST_{STHA} and HST_{MTHA}). Where the assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimate. Where significant outcomes were present, post-hoc tests with Bonferroni corrections were performed. The alpha level was $P < 0.05$. Data are presented as mean ± SD.

6.3. Results

6.3.1. Physiological and perceptual data

The physiological and perceptual data from this study is presented in Chapter 5. Briefly, resting T_{re}, T_{body} and TS were lower after HST_{STHA} and HST_{MTHA}, compared to HST_{PRE} (all $P < 0.05$), but no differences were observed in resting HR, T_{sk} and TC between all HSTs (all $P > 0.05$). Mean exercising physiological measurements HR, T_{re}, T_{sk} and T_{body} were higher during HST_{PRE} compared to HST_{STHA} (all $P < 0.026$) and HST_{MTHA} (all $P < 0.005$) but no differences were observed between HST_{STHA} and HST_{MTHA} (all $P > 0.05$). At the end of exercise both T_{sk}, T_{body} and HR were lower in HST_{STHA} and HST_{MTHA} compared to HST_{PRE} (all $P < 0.05$), where T_{re} was only lower after HST_{MTHA} ($P < 0.001$) but no differences in end of exercise physiological strain between HST_{STHA} and HST_{MTHA} (all $P > 0.05$). Sweat rate was similar in HST_{PRE} and HST_{STHA} ($P > 0.99$), but higher during HST_{MTHA} than HST_{PRE} ($P < 0.001$) and HST_{STHA} ($P < 0.001$). Fluid consumption was similar in all trials (main effect: $P = 0.827$) and the percentage of dehydration differed (main effect: $P < 0.001$), being greater in HST_{MTHA} compared to both HST_{STHA} ($P < 0.001$) and HST_{PRE} ($P < 0.001$).

6.3.2. Choice reaction time data - Table 6.1

6.3.2.1. Response time

There was an overall main effect for trial (P = 0.033) where response time was slower pre-exercise in HST_{STHA} compared to pre-exercise HST_{PRE} and response time was overall faster post-exercise (P = 0.004). There was no trial by time interaction effect (P = 0.060) (Figure 6.4).

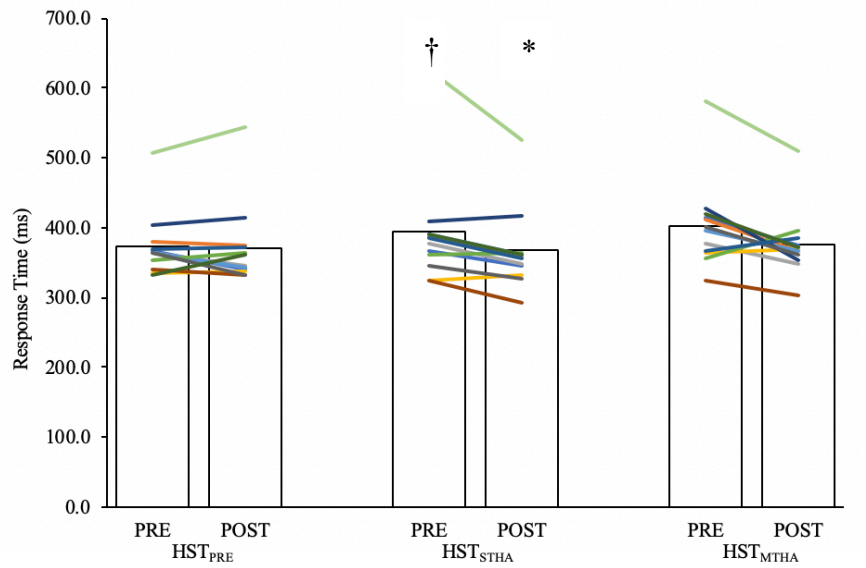

Figure 6.4. Mean and individual response time pre- and post-HST_{PRE}, HST_{STHA} and HST_{MTHA} (n = 12). *Significant (P < 0.05) difference within trial. †Significant (P < 0.05) difference between trials.

6.3.2.1.1. Acute heat exposure and HA

Acute heat exposure did not affect response time pre- to post-exercise during HST_{PRE} (0.6 ± 5.3%, P = 0.795). After five days participants responded faster post-exercise compared to pre-exercise during HST_{STHA} (6.1 ± 7.9%, P = 0.010) and after ten days during HST_{MTHA} (6.2 ± 7.9%, P = 0.019).

After five days of HA (HST_{STHA}) participants had a similar response time pre-exercise compared to pre-exercise response time in HST_{PRE} (P = 0.375) but had a slower response time pre-exercise after ten days HST_{MTHA} (P = 0.015) compared to HST_{PRE}. No differences between pre-exercise response time measurements from HST_{STHA} to HST_{MTHA} (P = 0.501).

After 45 min of exercise no differences in response time were revealed after five (HST_{STHA}; P = 0.541) or ten days (HST_{MTHA}; P > 0.99) of HA compared to HST_{PRE}, nor between five and ten days (P = 0.692).

6.3.2.2. Movement time

There were no main effects for trial (P = 0.163), time (P = 0.103) or trial by time interaction effect (P = 0.160) in the movement time taken to touch the stimulus after the button had been released.

Table 6.1. Mean ± SD for response and movement time pre- and post-heat stress tests (HST) (n = 12).

	Response time (ms)		Movement time (ms)	
	Pre-exercise	Post-exercise	Pre-exercise	Post-exercise
HST_{PRE}	372.9 ± 47.0	371.3 ± 59.4	183.8 ± 26.4	208.4 ± 46.3
HST_{STHA}	390.9 ± 78.5	365.2 ± 57.9*	194.7 ± 44.2	194.0 ± 40.8
HST_{MTHA}	403.1 ± 63.8†	375.6 ± 47.9*	205.7 ± 45.1	215.1 ± 47.1

*Significant ($P < 0.05$) difference within trials. †Significant ($P < 0.05$) difference compared to HST_{PRE}.

6.3.3. Spatial working memory (SWM)

6.3.3.1. Four boxes

When comparing the errors that occurred when four boxes were presented there were no differences between (all $P > 0.05$) or within trials (all $P > 0.05$).

6.3.3.2. Six boxes

There was a main effect for trial ($P < 0.002$) but not time ($P = 0.630$) nor was there an interaction effect ($P = 0.148$). Participants made less errors during HST_{MTHA} before the onset of exercise compared to HST_{PRE} ($P = 0.049$), but no differences were revealed between trials post testing between trials (all $P > 0.05$). There was no main effect in the number of times a box was revisited for trial ($P = 0.533$), time ($P = 0.438$) or

interaction effect (P = 0.533) when participants were presented with six boxes.

6.3.3.3. Eight boxes

When the number of boxes increased to eight, there was a main effect for trial (P < 0.001) but not time (P = 0.616) or interaction effect (P = 0.726). Participants performed more errors overall in HST_{PRE} compared to HST_{STHA} (P < 0.001) and to HST_{MTHA} (P = 0.011). These errors occurred before the onset of exercise, where participants performed less errors in HST_{STHA} (P = 0.013) and HST_{MTHA} (P = 0.049) compared to HST_{PRE}. No differences existed between HST_{STHA} and HST_{MTHA} (P > 0.99).

After exercise, a greater number of errors were made during HST_{PRE} compared to HST_{STHA} (P = 0.003). No differences were revealed between HST_{PRE} and HST_{MTHA} (P = 0.069) or between HST_{STHA} and HST_{MTHA} (P > 0.99). There was no main effect for the number of times a box was revisited for trial (P = 0.181), time (P =0.499) or interaction effect (P = 0.123).

6.3.3.4. Twelve boxes

When the greatest number of boxes were presented, there was a main effect for trial (P = 0.003) but not time (P = 0.274) or interaction effect (P = 0.564). Participants performed more mean errors overall in HST_{PRE}

compared to HST_{MTHA} (P = 0.010) but no difference to HST_{STHA} (P = 0.113) or between HST_{STHA} and HST_{MTHA} (P = 0.617).

Before the onset of exercise participants had less errors in HST_{MTHA} compared to HST_{PRE} (P = 0.024); however, there were no other differences between trials pre- or post-exercise when presented with twelve boxes (all P > 0.05) (Figure 6.5). There was no main effect for the number of times a box was revisited for trial (P = 0.888), time (P =0.396) or interaction effect (P = 0.398).

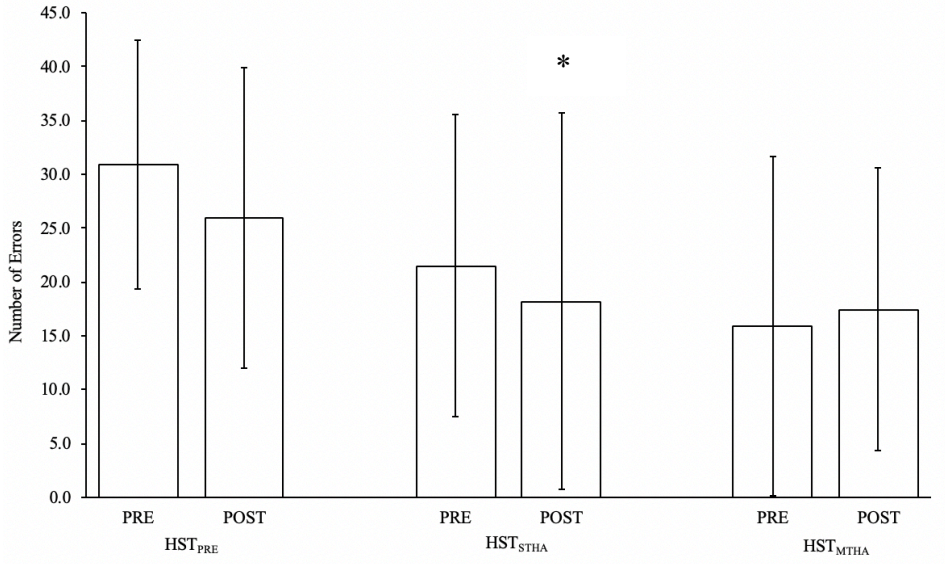

Figure 6.5. The number of errors performed when presented with 12 boxes (n = 12). The bar represents the mean data value. *Significant (P < 0.05) difference to HST_{PRE}.

6.4. Discussion

The aim of the present study was to investigate whether an acute bout of exercise when heat stressed had any effect on cognitive function and whether a daily 60 min isothermic short-term (five days) and medium-term

(ten days) HA protocol had any effect on cognitive function from baseline measurements.

6.4.1. Acute exercise to heat stress

6.4.1.1. Choice reaction time responses

The main findings were that an acute bout of exercise under high thermal strain did not impair simple or complex cognitive function in endurance trained participants. There were no differences found in response or movement time post-exercise, where physiological and perceptual strain were the highest during the first exposure to heat stress. These findings are in contrast with previous studies that found a rise in core temperature above a thermal strain (>38.7 ºC) to be associated with a decline in reaction time (Gaoua *et al.*, 2011). It was, therefore, surprising and in contrast to previous findings to report response and movement time to not be impaired post-exercise during HST_{PRE} compared to pre-exercise measurements, as participants had a high T_{re} of 38.94 ± 0.51 °C. While unable to determine the exact mechanism to underpin this unaltered reaction time response it may be at least partially explained by a hyperthermia-induced enhancement in neural transmission (Piil *et al.*, 2017) and nerve conduction velocity (Racinais, Gaoua and Grantham, 2008).

6.4.1.2. Spatial working memory response

Another main finding in the present study was that acute heat stress impaired short-term memory when task complexity was increased (i.e.

when participants were presented with eight and twelve boxes). Acute heat stress had no effect when task complexity was lower and participants were presented with fewer boxes (four and six boxes). Previous evidence and data from the present study support that the influence of heat stress on cognition is task-complexity dependant (Gaoua *et al.*, 2011; Piil *et al.*, 2017). While not measured in the present study, this response could be explained by the physiological alteration of the brain during hyperthermia (Nielsen *et al.*, 2001; Gaoua *et al.*, 2011). It is possible that the greater neuronal demand that complex tasks require and the additional strain that hyperthermia imposes on the brain combine to provide a greater cognitive load, draining cognitive resources and increasing mental fatigue (Cian *et al.*, 2001; Gaoua *et al.*, 2018).

6.4.2. Short-term and medium-term HA

6.4.2.1. Reaction time response

Short-term HA had neither a positive nor a negative effect on cognitive function compared to pre-acclimation testing but after MTHA participants responded more slowly during pre-exercise testing compared to pre-acclimation. Even though this slower response time was not significant after STHA, this response after MTHA was likely due to the adaptations that occurred to the thermoregulatory system. We observed that after ten days of HA, participants rated a lower TS and had a lower resting T_{re} (36.69 ± 0.24 ºC) compared to HST_{PRE} (37.09 ± 0.23 ºC) but no other differences were found between trials before the onset of exercise. Faster

reaction times have been reported to occur when both T_{sk} and T_{re} are elevated in combination (observations supported by Simmons *et al.* (2008b)) whereas, decrements in cognitive performance are apparent when T_{re} increases alone. The current data presented, makes it difficult to identify at which rectal temperature decrements to reaction time occurred; however, a high T_{re} (>38.55 °C) had neither a positive nor negative effect on reaction time response in the present study. It is important to highlight that a slower reaction time does not always translate to impaired cognitive function, whereby accuracy of responses has been shown to improve when reaction time is slower (Simmons *et al.*, 2008).

Despite response time post-exercise after STHA and MTHA was faster, these responses were not improvements from pre-acclimation cognitive function, over the time-course of the HA protocol participants appeared to react to a stimulus more slowly pre-exercise. Sensations of fatigue are a complex issue and far less is known on the influence of a HA protocol on these feelings, there is uncertainty whether consecutive repeated days of HA may have increased sensations of fatigue (Willmott *et al.*, 2019). As cumulative fatigue was not directly measured in the present study, it is uncertain to whether the slower reaction time at baseline was also due to an increased sensation of fatigue.

An increased ability to react more quickly to a stimulus is highly beneficial to athletes in endurance sports, such as a cyclist having to react to other cyclists in a peloton to avoid collisions or being able to respond to

unexpected obstacle (i.e. rock, uneven surface) during running. Within the present study reaction time was increased within trials after five and ten days of HA but not different between trials. Gaoua *et al.* (2018) reported that passive hyperthermia to increased speed response, which the authors suggest that this could possibly due to an increase in nerve conduction velocity and impulsivity. In present study, we are unable to confirm previous observations of Gaoua *et al.* (2018) as electroencephalography was not measured during cognitive function tasks.

6.4.2.2. Spatial working memory

The data show that STHA and MTHA was more beneficial to complex task, whereas fewer errors made during the spatial working memory test as task complexity increased than compared to pre-acclimation (HST_{PRE}). One of the primary adaptations to a longer exposure duration of HA (e.g. MTHA) is a lower thermoregulatory strain at rest, during and end of exercise and, with this in mind, it is possible that the lower thermal strain associated with MTHA can account for the fewer errors made during the most complex special working memory test. This improvement was not seen at the midway point (STHA) nor when the spatial working memory task was simplified (i.e. 4 vs. 12 available boxes). This response was likely due to cognitive resources being insufficient for the cognitive task as both thermal strain and task complexity imposes a greater cognitive load (Hocking *et al.*, 2001). While not directly measured in the present study, electroencephalography has been recorded during cognitive function tasks

during passive exposure to the heat, providing insight into the brain activity that occurs. During cognitive function testing under heat stress alpha activity decreases but theta activity increases suggesting that during heat stress greater concentration and attention is required. The increased concentration and attention may impose a greater mental workload demand and subsequently increase cognitive fatigue (Smith, Eling and Coenen, 2004; Gaoua *et al.*, 2018).

6.4.3. Considerations and potential limitations

Although this study was not designed to investigate the effects of hydration status on cognitive function, it was found that participants had a greater percentage of body mass loss after ten days of HA (>2%) than compared to pre-acclimation and five days of HA (<2%). Previous findings have identified mild hypohydration to impair cognitive function (Cian *et al.*, 2000; Kempton *et al.*, 2011), in contrast the present study showed that mild hypohydration had neither a positive nor a negative effect on cognitive function during heat exposure. Furthermore, it should be noted that due to a lack of control group used in the present study, the possibility of a learning effect cannot be excluded. In addition, there are further potential limitations to be considered including a lack of sensitivity of the cognitive function test and the short duration of the test used within the current study.

6.5. Conclusion

The data from the present study showed that an acute bout of exercise under heat stress did not impair cognitive function in endurance trained individuals and that a 60 min daily isothermic HA protocol over five (STHA) and ten days (MTHA) did not improve cognitive function from baseline measurements but improved within trial measurements. The results from the present study reject all three research hypotheses (H_1-H_3). A MTHA protocol protected cognition when task complexity was increased but had no effect on reaction time responses. Improved complex task performance would be especially important to athletes whose sport involve reacting to hitting a ball (i.e. tennis), team strategies and reacting to a potential obstacle that may cause injuries (Jiménez-Pavón *et al.,* 2011). The next study addresses the lipopolysaccharide (LPS) response after an acute bout of exercise performed under heat stress and after a STHA and MTHA isothermic HA protocol.

Chapter 7. **The Effects of a Short-term and Medium-term Daily Isothermic Heat Acclimation Protocol on the Lipopolysaccharide (LPS) Response to Exercise under Heat Stress**

7.1. Introduction

Exercising in the heat imposes a greater physiological challenge to the thermoregulatory and cardiovascular systems than compared to exercising in temperate conditions (González-alonso, Crandall and Johnson, 2008; Sawka *et al.*, 2011; Nybo, Rasmussen and Sawka, 2014). The primary cardiovascular challenge during exercise in the heat is to meet the thermoregulatory demands of blood flow to the skin to facilitate heat loss, while providing sufficient blood flow to the skeletal muscles to support the energetic demands of ATP production and metabolic clearance (Selkirk *et al.*, 2008). This redistribution of blood flow negates an increase in heart rate to maintain cardiac output (Nadel, 1979). One region from which blood is redistributed is the splanchnic region and it has been reported that there are declines of up to 80% in splanchnic blood flow (Rowell, 1973, 1974; Otte *et al.*, 2001). Potentially, as a result of splanchnic hypoperfusion during exercise, which may lead to ischaemic injury to the gut, the integrity of the intestinal epithelial cell walls is impaired (Pals *et al.*, 1997; van Wijck *et al.*, 2011) and this, in combination with the thermal load further promotes epithelial damage or dysfunction occurring to the gastrointestinal (GI) tract (Moseley and Gisolfi, 1993; Hall *et al.*, 1999; Gill *et al.*, 2015).

The epithelial lining along the GI tract acts as a protective barrier that separates the internal aseptic environment of the circulatory system from the external septic environment within the gut (Lambert *et al.*, 2002; Gill

et al., 2015; Dokladny, Zuhl and Moseley, 2016). When the integrity of the GI tract is compromised, a translocation of endotoxins (e.g. lipopolysaccharide (LPS)) can diffuse from the intestinal lumen into the internal environment (Lambert, 2008). Small amounts of intestinal permeability occur within healthy individuals and low levels of endotoxins are cleared rapidly by hepatic macrophages, known as Kupffer cells, as part of an innate immune response (Treon, Thomas and Broitman, 1993; Shao *et al.*, 2012). Damage to the intestinal epithelial cells results in an increase in permeability, allowing for a greater influx of bacteria (e.g. endotoxins, food antigens, digestive enzymes and bile) to translocate from the intestinal lumen in to the portal and systemic circulation (Lim *et al.*, 2009; Armstrong, Lee and Armstrong, 2018). The translocation of the gram-negative bacteria LPS (an endotoxin from the outer membrane) triggers a cascade of pro- and anti-inflammatory cytokine responses (e.g. TNF-α, IL-1 and IL-6), which can further increase core temperature, exacerbate the symptoms of heat stroke and increase symptoms of fatigue (Lim and Mackinnon, 2006; Lim *et al.*, 2009; Vargas and Marino, 2016).

There is growing evidence that supports the complex interplay of both heat and exercise-induced endotoxemia in the aetiology of exertional-heat illness (Moseley and Gisolfi, 1993; Lim, 2018). The highest circulating LPS concentrations have been reported after prolonged exercise performed in hot environmental conditions when core temperatures are elevated (Selkirk *et al.*, 2008; Gill *et al.*, 2015a; 2015b). For example, in one study,

where blood samples were drawn at different rectal temperatures (baseline, 38 ºC, 38.5 ºC, 39 ºC & 39.5 °C) during exercise in the heat (40 °C), endotoxin levels were observed to be higher from baseline at a rectal temperature of 38.5 °C onwards (Selkirk *et al.*, 2008). Elevated concentrations of LPS associated with exercise performed in the heat have been associated with more pronounced symptoms of GI distress including vomiting, nausea, diarrhoea and sensations of fatigue, which subsequently impairs exercise performance (Bosenberg *et al.*, 1988; Jeukendrup *et al.*, 2000). The relationship between increased intestinal permeability and GI complaints resulting in systemic endotoxemia has been shown in individuals with heat stroke (Costa *et al.*, 2017) and in runners who collapsed during an ultra-marathon competition in the heat (Brock-Utne *et al.*, 1988).

Limited evidence currently exists on interventions to prevent GI permeability and endotoxemia during exercise in the heat. To date, the literature has primarily focused on the use of nutrition and supplementation interventions, yet there is still a lack of understanding (Guy and Vincent, 2018). Probiotic supplementation may help to maintain GI integrity by enhancing the stability of tight junctions between the epithelial cells via an increased secretion in mucin, immunoglobin A and an improvement in overall gut microbiota (Shing *et al.*, 2014; Taira *et al.*, 2015). Shing *et al.* (2014) reported that four weeks of supplementation prior to an exercise capacity test in the heat (35 ºC to 40 °C) reduced post-

exercise circulating LPS concentrations. In contrast, non-steroidal anti-inflammatory drugs (NSAIDs) (e.g. ibuprofen), which are commonly used to reduce pain during exercise, can increase intestinal damage and therefore, increase the translocation of LPS into the internal environment (Van Wijck *et al.*, 2012).

Repeated exposure to artificially hot environmental temperatures, a process known as heat acclimation (HA), can elicit many physiological adaptations that reduce thermoregulatory and cardiovascular strain (refer to section 2.8) (Périard, Racinais and Sawka, 2015), which may offer protection to the GI barrier (Vargas and Marino, 2016). Up to 80% of these adaptations occur within the first seven days of heat exposure (short-term heat acclimation (STHA)), however, the magnitude of adaptation appears to be greater with medium-term (MTHA; 7 days to 14 days) and long-term HA (LTHA; >15 days) (Tyler *et al.*, 2016). HA-induced hypervolemia decreases the cardiovascular strain at a given exercise intensity and an earlier onset of sweating may facilitate heat loss and therefore, attenuate the rate of rise in core temperature (Sawka, Pandolf, *et al.*, 1983). Both mechanisms may potentially reduce or delay, the exercise-induced reduction in splanchnic blood flow and thermal load that results in an increased intestinal permeability (Lambert, 2004), during heat stress. In recent years, there is limited research that has investigated the effects of STHA as a protective intervention to reduce the exercise-induced endotoxin leakage (Kuennen *et al.*, 2011; Barberio *et al.*, 2015; Guy *et al.*,

2016). To date, the literature investigating the effect of HA on exertional-endotoxemia is currently inconclusive (Kuennen *et al.*, 2011; Barberio *et al.*, 2015; Guy *et al.*, 2016), with methodological issues and inconsistencies arising from the studies referenced.

For heat adaptations to occur, heat stress must induce a physiological strain of a magnitude above an adaptation threshold (Taylor, 2014) and the magnitude of the adaptation appears dependent on the extent and frequency of thermal strain and thermal impulse provided. LPS responses appear to be associated with the type, intensity and duration of exercise, because while neither Kuennen *et al.* (2011) nor Guy *et al.* (2016) observed any changes in circulating LPS concentrations during and after a low intensity cycling exercise protocol for ~40 min to 45 min (~55% $\dot{V}O_{2max}$), Barberio *et al.* (2015) reported within session elevations following higher intensity (~78% $\dot{V}O_{2max}$) running in the heat until exhaustion or a set increase in core temperature (+2 °C). These data suggest that one factor required to observe a measurable LPS response is to exceed a physiological strain above a certain threshold. However, this threshold is currently unknown and none of these studies have observed a beneficial effect of STHA (<7 days) on LPS response. In the studies by Kuennen *et al.* (2011) and Guy *et al.* (2016), this is unsurprising because they did not observe a within session increase in LPS. Controversially, Barberio *et al.* (2015) reported that this response was not attenuated by HA, possibly because the STHA protocol investigated provided a

cumulative physiological strain below the theorised threshold. Barberio *et al.* (2015) investigated a five day HA protocol using a set increase in core temperature (+2 °C) and a potential limitation to using this, is that as adaptation occurs and resting core temperature lowers, individuals may not be reaching a sufficient thermal strain relative to the start of HA and/or the HA regimen may not have been long enough to induce adaptation. Therefore, it is yet to be concluded whether there is a cumulative strain rather than an acute physiological strain that is linked to the translocation of LPS into the systemic circulation during exercise in the heat, resulting in the development of exertional heat illness.

Therefore, the primary aim of the present study was to investigate whether an acute bout of exercise under high thermal strain would induce the endotoxin LPS response to when exercising in the heat in moderately-trained endurance individuals. The secondary aim was to determine if the endotoxin LPS response to occur from the first heat exposure session would decline after a daily STHA isothermic protocol (target rectal temperature ~38.5 ºC) and whether MTHA was more effective than STHA.

It was hypothesised that:

H_{01}: an acute bout of exercise under heat stress has no effect on LPS concentrations pre- to post-HST.

H_1: an acute bout of exercise under heat stress increases LPS concentrations when measured from pre- to post-HST.

H_{02}: a daily 60 min isothermic HA protocol would have no effect on circulating endotoxin LPS concentrations.

H_2: a daily 60 min isothermic HA protocol would reduce circulating endotoxin LPS concentrations.

H_3: this response would be greater following MTHA than STHA, where MTHA would potentially offer more complete adaptations to the cardiovascular and thermoregulatory systems.

7.2. Methods

7.2.1. Participants

The data presented in this experimental chapter were collected during the data collection of Chapter 5. Blood samples were taken from nine out of the sixteen, non-heat acclimated, endurance runners (females = 2) (mean ± SD: age 39.0 ± 9.7 y, stature 177.6 ± 2.5 cm, body mass 76.6 ± 9.2 kg, body fat percentage 10.7 ± 4.9% and maximal workload (W_{max}) 301 ± 76 W) who participated, due to time restrictions and individual preferences to not have blood drawn. Before participation, all participants completed a health screening questionnaire and provided written fully informed consent to participate. The study was approved by the University of Roehampton's

ethical committee (LSC 18/228 - Appendix C3) and all procedures and protocols adhered to the guidelines of the World Medical Association (*Declaration of Helsinki*). Data were collected between March and April (mean outside temperature ~6 °C) in the United Kingdom to avoid heat acclimatisation. For calibration measurements of equipment used see Appendix E1.

7.2.2. Experimental procedures (Figure 7.1)

Full experimental details are presented in Chapter 5. In brief, participants undertook one preliminary visit (for assessment of maximal power output) and thirteen consecutive experimental visits. Participants performed a 45 min sub-maximal (40% W_{max}) heat stress test (HST) on the first (HST_{PRE}), seventh (HST_{STHA}) and thirteenth (HST_{MTHA}) experimental visits. Between HST_{PRE} and HST_{STHA} and again between HST_{STHA} and HST_{MTHA}, participants completed five consecutive days of isothermic HA (60 min each visit) during which time rectal temperature (T_{re}) was elevated to, and maintained at ~38.5 °C. The environmental conditions were 40 °C and 50% relative humidity (rh) with no convective cooling applied for all sessions. Blood samples were collected pre- and immediately post- each HST (Figure 7.1). Participants were instructed to avoid caffeine, alcohol, and strenuous exercise 24 h before all HSTs. HSTs were performed at the same time of day for each participant throughout the study to avoid the effects of circadian rhythm. Food intake was recorded for the 24 h prior to

HST_{PRE} and participants were instructed to replicate this before HST_{STHA} and HST_{MTHA}.

7.2.3. Plasma lipopolysaccharide sample collection and analyses

Venous blood was drawn from nine participants pre- and post-HST, using a 21-gauge blood safety collection kit (Greiner Bio-One International, GmbH, Kremsmünster, Austria) from a prominent superficial forearm vein located at the antecubital fossa, and drained directly into a sterile 9 mL K3EDTA tube (Greiner Bio-One International, GmbH, Kremsmünster, Austria) before being centrifuged at 3000 rpm for 10 min at 4 °C. Plasma was extracted using pyrogen-free pipette tips into pyrogen-free microtubes (Eppendorf, Hamburg, Germany) before being frozen at -80 °C. Plasma concentrations of LPS were analysed using a high-sensitivity (detection range 0.04 to 10.0 $EU \cdot mL^{-1}$) chromogenic limulus amoebocyte lysate (LAL) end-point assay kit (Hycult Biotechnology b.v., Uden, Netherlands). Plasma samples were thawed and brought to room temperature before being diluted by 1000 times with endotoxin-free water. Fifty microlitres of each sample were then transferred into the wells of pyrogen-free microplate in duplicates, followed by 50 µL of LAL reagent. Optical density of the reaction was read with a microplate reader (Thermo Scientific Multiskan EX) at a wavelength of 405 nm.

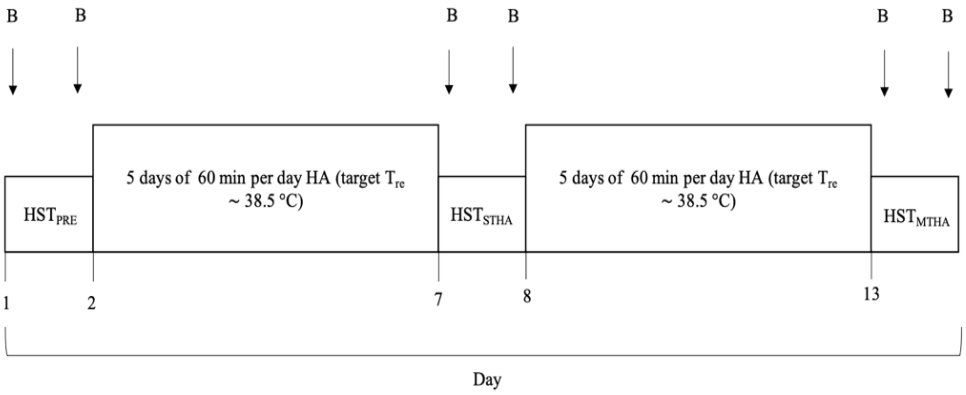

Figure 7.1. Schematic design of experimental protocol. Blood samples (B) were drawn pre and post heat stress test (HST) on the first (HST_{PRE}), seventh (HST_{STHA}) and thirteenth (HST_{MTHA}) visits. Full details of experimental protocol are presented in Chapter 5.

7.2.4. Statistical analyses

Data were analysed using SPSS (version 26, SPSS Inc.). Parametric assumptions were met unless stated otherwise. A one-way and a two-way repeated measures ANOVA were performed to determine differences between pre- and post- time points within trials and between trials in HST_{PRE}, HST_{STHA} and HST_{MTHA}. Where the assumption of sphericity was violated, the degrees of freedom were corrected using the Greenhouse-Geisser estimate. Where significant outcomes were present, post-hoc tests with Bonferroni corrections were performed. The alpha level was $P < 0.05$. Data are presented as mean ± SD.

7.3. Results

7.3.1. Physiological and perceptual responses

The physiological and perceptual data from this study are presented in Chapter 5. To summarise, resting T_{re}, T_{body} and TS were lower after HST_{STHA} and HST_{MTHA}, than compared to HST_{PRE} (all $P < 0.05$), but no differences were observed in resting HR, T_{sk} and TC between all HSTs (all $P > 0.05$). Mean exercising physiological measurements HR, T_{re}, T_{sk} and T_{body} were higher during HST_{PRE} compared to HST_{STHA} (all $P < 0.026$) and HST_{MTHA} (all $P < 0.005$) but no differences were observed between HST_{STHA} and HST_{MTHA} (all $P > 0.05$). At the end of exercise, HR, T_{sk}, and T_{body} were lower in HST_{STHA} and HST_{MTHA} compared to HST_{PRE} (all $P < 0.05$) and T_{re} was lower after HST_{MTHA} compared to HST_{PRE} ($P < 0.001$). There were no other physiological differences at the end of exercise between HST_{STHA} and HST_{MTHA} (all $P > 0.05$).

7.3.2. Lipopolysaccharide response

Plasma LPS concentrations were similar between trials before (HST_{PRE}: 1.46 ± 0.57 $EU \cdot mL^{-1}$; HST_{STHA}: 1.49 ± 0.54 $EU \cdot mL^{-1}$; HST_{MTHA}: 1.52 ± 1.36 $EU \cdot mL^{-1}$, $P = 0.926$) and after 45 min of exercise (HST_{PRE}: 2.00 ± 1.65 $EU \cdot mL^{-1}$; HST_{STHA}: 1.74 ± 0.85 $EU \cdot mL^{-1}$; HST_{MTHA}: 1.72 ± 0.82 $EU \cdot mL^{-1}$, $P = 0.869$). The mean increase observed during each HST was 0.54 ± 1.17 $EU \cdot mL^{-1}$, 0.25 ± 1.01 $EU \cdot mL^{-1}$ and 0.05 ± 0.38 $EU \cdot mL^{-1}$, for HST_{PRE}, HST_{STHA} and HST_{MTHA}, respectively ($P = 0.420$) (Figure 7.2).

There was a large degree of variation in the percentage change within trials in HST_{PRE} (+50% to 218%), HST_{STHA} (+49% to 213%), and HST_{MTHA} (+29% to 402%) but LPS concentrations increased in all participants in all trials.

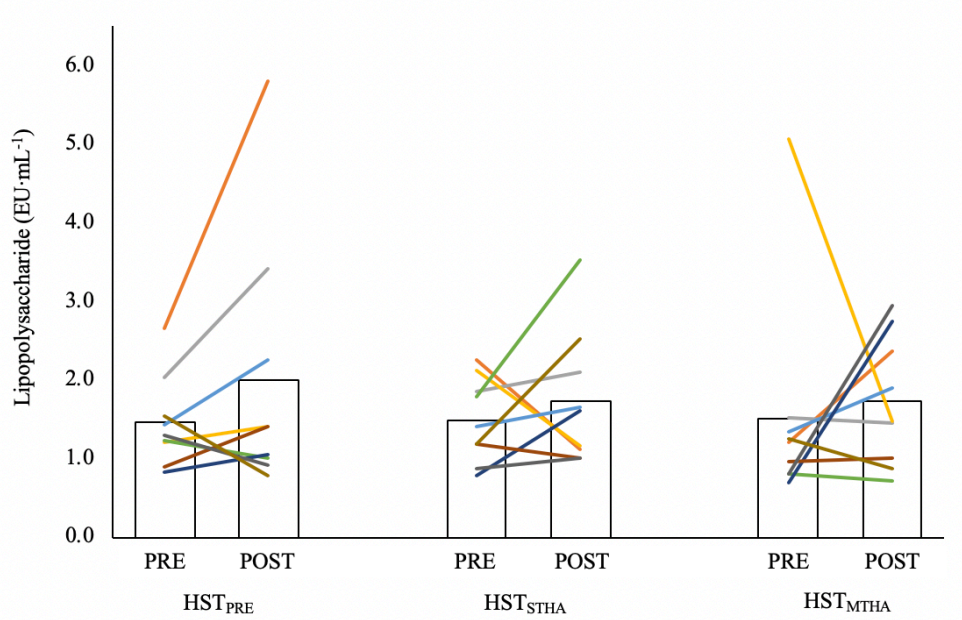

Figure 7.3. Mean and individual data lines for lipopolysaccharide (LPS) response pre- and post-HSTs for HST_{PRE}, HST_{STHA} and HST_{MTHA} (n = 9).

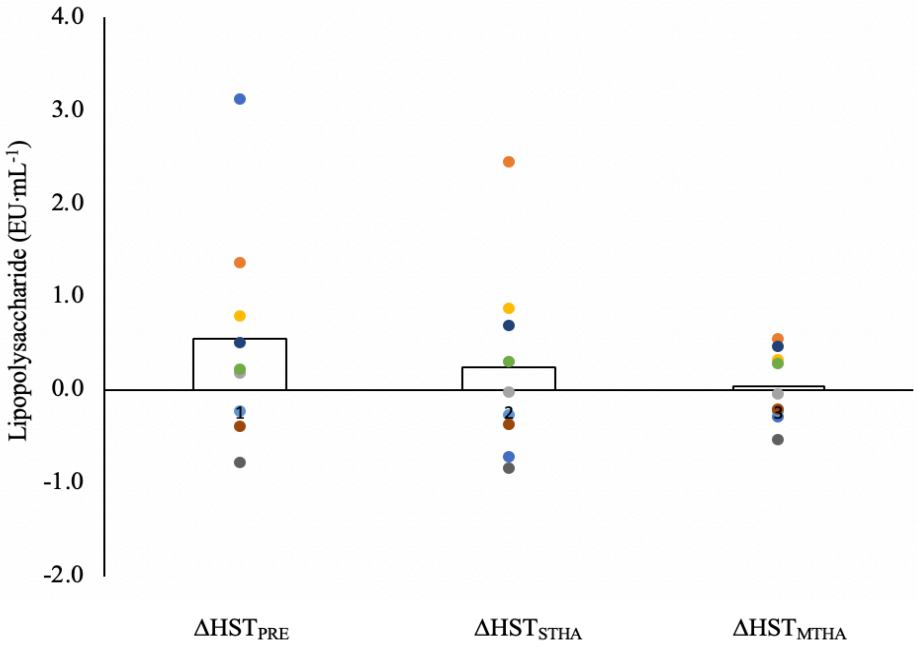

Figure 7.2. Mean and individual data points for delta (Δ) change in lipopolysaccharide (LPS) response pre- and post-HST_{PRE}, HST_{STHA} and HST_{MTHA} (n = 9).

7.4. Discussion

The present study aimed to investigate whether a daily 60 min isothermic HA protocol had any effect on the within-trial endotoxin LPS responses over a five (STHA) and a ten day (MTHA) time-course. The main findings were that neither STHA nor MTHA had an effect on the circulating LPS response to HA. Thus, no differences were observed from pre- to post-HST at any point. The whole-body physiological and perceptual data are reported in detail in Chapter 5. In brief, the daily 60 min isothermic STHA protocol provided a sufficient cumulative thermal strain to effectively lower physiological and perceptual strain and MTHA induced further beneficial heat adaptations to total sweat losses, final thermoregulatory strain and perceptions of thermal strain and comfort.

It is well established that prolonged exercise performed in hot environmental conditions increases thermoregulatory and cardiovascular strain (González-alonso, Crandall and Johnson, 2008) and it is likely that it is this complex interplay of physiological strain that leads to intestinal damage and a translocation of LPS from the GI tract into the systemic circulation (Selkirk *et al.*, 2008), potentially provoking a systemic immune reaction (Flynn and McFarlin, 2006) mediated by the central nervous system (CNS) (Lynch *et al.*, 2003). In contrary to the above, our data showed that despite an increase in cardiovascular and thermoregulatory strain post-HST_{PRE}, the methods used did not increase circulating plasma concentrations of LPS. In addition to this, as thermal adaptations occurred

from the isothermic HA protocol, no differences were observed in pre- to post-plasma LPS concentrations after five (STHA) and ten (MTHA) days of heat exposure. In accordance with our findings, both Kuennen *et al.* (2011) and Guy *et al.* (2016) reported that after STHA (five days) there was no response in LPS concentrations from pre- to post-HST. However, in another STHA study (seven days), circulating LPS concentrations were elevated post-exercise, after participants ran at ~78% of their $\dot{V}O_{2max}$ until exhaustion or until core temperature had increased by 2 °C (Barberio *et al.*, 2015).

The discrepancies reported between these studies might be explained by the mode of exercise used. In the current study and as reported by Kuennen *et al.* (2011) and Guy *et al.* (2016) cycling was used whereas Barberio *et al.* (2015) used a more intensive running protocol. Running may elicit greater gut trauma due to a greater mechanical bounce occurring, which may compromise GI barrier function and thereby, increases the translocation of LPS into the systemic circulation (Costa *et al.*, 2017), as suggested by Barberio *et al.* (2015).

The participants used in the present study were moderately-trained ultra-endurance runners, who were about take part in the Marathon des Sables, a 250 km foot-race across the Sahara Desert. It has been proposed that through training and therefore, potentially more frequent exposure to endotoxin release, individuals have an enhanced resistance and resilience to circulating LPS concentrations (Brock-Utne *et al.*, 1988; Gill *et al.*,

2015). Where trained individuals have a significantly higher anti-inflammatory response compared to untrained individuals (McFarlin *et al.*, 2006). One of the primary signal receptors for LPS, is the Toll-like receptor 4 (TLR-4), which has been shown to be lower in trained individuals (Flynn and McFarlin, 2006; McFarlin *et al.*, 2006), however, in the present study we did not measure this and are unable to determine if there was an anti-inflammatory and TLR-4 response. Training status has also been shown to influence the redistribution of splanchnic blood flow, where untrained individuals experience a greater reduction in splanchnic blood flow compared to trained individuals (Rowell, 1973). This reduction in splanchnic blood flow results in an earlier and greater influx of endotoxins into the portal and systemic circulation (Gill *et al.*, 2015). It appears that exercise intensity affects the magnitude of increase in LPS concentrations post-exercise, where an elevated LPS response was reported after a high intensity exercise protocol (Barberio *et al.*, 2015), in comparison to the present study, which used a low fixed-intensity of 40% W_{max}. In one study, Pals and co-authors (1997) investigated the effects of running intensity on intestinal permeability and reported that running at 80% of $\dot{V}O_{2max}$ increased intestinal permeability compared to when at rest, exercising at 40%, and at 60% of $\dot{V}O_{2max}$. Therefore, exercise intensity and training status of the individuals might explain the insignificant circulating LPS response post-exercise in the present study, as intestinal permeability was unlikely to be increased and therefore, endotoxins were unable to cross the intestinal epithelia.

Furthermore, the present study showed that five and ten consecutive days of exercising in the heat did not elicit a sustained rise in circulating LPS concentrations when measured from post-HST_{PRE} to pre-HST_{STHA} and from post-HST_{STHA} to pre-HST_{MTHA}. This conflicts with previous literature, that measured the endotoxin concentrations of ultra-endurance runners throughout a multi-stage ultra-marathon (Gill *et al.*, 2015). Gill *et al.* (2015) reported that despite overnight recovery between running stages, runners sustained an elevated LPS concentration throughout the race. The lack of change or sustained elevation in LPS concentrations shown in the present study may indicate that the use of an isothermic HA protocol is highly tolerable for individuals without the translocation of LPS into the circulation, while eliciting the necessary thermal adaptations to reduce thermal strain during exercise in the heat.

7.4.1. Considerations and potential limitations

Unlike Guy *et al.* (2016) and Selkirk *et al.* (2008), the anti-inflammatory cytokine response was not measured in the present study and therefore, we are unable to exclude the possibility that there was an increase in circulating inflammatory cytokine response over five and ten days of HA. Within the literature available, a variety of methodologies have been used to measure LPS concentrations, that have varied levels of sensitivity, which might possibly explain the differences reported across the literature. Furthermore, the type of exercise used has predominately been running protocols, which elicits higher levels thermal strain (Barberio *et al.*, 2015),

in addition to different training statuses of individuals. It has been documented that high core temperatures of >41.5 °C results in an increase in intestinal permeability leading to LPS entering the systemic circulation. While unethical within a research setting to exercise participants to this level of thermal strain, the overall mean highest rectal temperature reached in the present study was during the first HST (HST_{PRE}; 38.94 ± 0.51 °C). Furthermore, in the present study the participants' diet was standardised for 24 h but was not manipulated nor was supplementation intake controlled and therefore, we are unable to comment on whether nutrition or supplementation played a role in the LPS response. There is evidence to support that consuming a high-fat diet is associated with an increase in circulating LPS concentrations (Pendyala, Walker and Holt, 2012). While unable to be certain, it is possible that our participants may have consumed a high carbohydrate diet, as they were about to undertake an ultra-endurance marathon race. The plasma cytokine response IL-6 is closely related to muscle glycogen content and therefore, the intake of a high carbohydrate diet which minimises the rate at which muscle glycogen is depleted, has been reported to lower IL-6 and LPS circulating concentrations post-exercise (Bishop *et al.*, 2001; Chan *et al.*, 2004; Snipe *et al.*, 2017). In addition to the above, potential limitations exist with the LPS test used within the present study, which include a low sensitivity and reliability of these tests.

7.5. Conclusion

Exercising in hot environmental conditions for 45 min at sub-maximal intensity did not increase circulating plasma concentrations of LPS after one bout of heat exposure or consecutive bouts of heat exposures after five (STHA) and ten days (MTHA). Furthermore, a daily 60 min isothermic HA protocol over a short- and medium-term time course did not elicit any further changes in circulating LPS concentrations. The results of the present study reject all three hypotheses (H_1-H_3). We cannot exclude the possibility that a more intense exercise protocol or a longer duration exercise bout would not lead to a greater circulating LPS response. Further research is warranted to investigate whether an isothermic HA intervention during a running protocol would increase circulating LPS concentrations.

Chapter 8. **General Discussion**

8.1. Introduction and key findings

This thesis examined the three key interventions that are proposed to offset the reductions in exercise performance in the heat while alleviating thermal strain.

The first investigation explored **the influence of perception and expectation of hydration status on cycling performance in the heat** (Chapter 3), with the second, investigating **the effects of external pre- and per-cooling in isolation and/or combination on cycling performance in the heat in highly trained participants** (Chapter 4). The final experiment presented in Chapters 5, 6 and 7 investigated **the effects of a daily 60 min isothermic HA protocol (target rectal temperature ~38.5 ºC) on the physiological and perceptual adaptations to exercise heat stress, cognitive function, and the LPS response to acute and chronic heat stress**.

8.2. Principle findings

8.2.1. The influence of perception and expectation of hydration status on cycling performance in the heat

The data from Chapter 3 demonstrated that perceived hydration status did not influence 15 min cycling TT performance in the heat in healthy individuals. The detrimental effects of hypohydration (≥2%) on exercise have been well-documented in laboratory-controlled studies (Cheuvront *et al.*, 2005; Stearns *et al.*, 2009; Logan-Sprenger *et al.*, 2015) but yet to be

established was to what extent was awareness of hydration status had on exercise performance. While the physiological mechanisms explaining how hypohydration might impair exercise capacity and performance are clear, a number of methodological limitations exist regarding how hypohydration is induced. To date, the use of the nasogastric tube has been used in limited previous studies (James *et al.*, 2017; Funnell *et al.*, 2019), which primarily focused on comparing performance outcomes when euhydrated and hypohydrated (~2%). In both the aforementioned studies, performance was impaired (-8% to 11%) when hypohydrated compared to when euhydrated and information obtained from the exit interviews disclosed that participants were unable to identify the true experimental aim. To the author's knowledge, the perception and expectation of hydration status on performance when blinded to the true aim of the study had not been previously investigated and therefore, this was the first study to date.

The results from Chapter 3 provide evidence to show that previous laboratory-controlled studies were not confounded by methodological limitations where participants were unlikely not blinded to the aim of the study (i.e. participants were aware of hydration status). Therefore, the impaired performance outcomes documented in these studies were likely due to the physiological consequences of hypohydration, rather than methodological limitations. Furthermore, the use of a nasogastric tube was a successful methodological approach to blind participants to their true hydration status as they were unable to identify the true aim of the study

when interviewed post-trial. The rationale for male participation was based upon the sex-related differences in body fat composition and gastrointestinal core temperature responses due to the menstrual cycle that would interfere with sweat rate response. Therefore, to reduce potential error when infusing fluid to match sweat losses female participation was excluded. The disadvantage of excluding female participation in this study therefore, does not allow for the conclusion to be made that the outcome of the study would be similar in female participants.

8.2.2. The effect of isolated and/or combined external pre- and per-cooling on cycling performance in highly trained cyclists/triathletes in the heat

Data in Chapter 4 show that neither pre- nor per-cooling in isolation or the combination of both interventions improved 15 min cycling performance in the heat in highly trained individuals. External whole-body CWI (22 ºC) was successful at lowering both physiological and perceived thermal strain before the onset of exercise, with a lower rectal temperature persisting throughout the 45 min preload. The benefits of pre-cooling on skin temperature and cardiovascular strain were transient in comparison to the longer lasting benefit on lowering rectal temperature throughout the preload. Cooling the neck region, via a neck cooling collar, was successful at lowering mean neck temperature but was unsuccessful at adding any additional benefit to these physiological and perceptual measurements when combined with pre-cooling or used in isolation during the preload exercise. The short-lived beneficial response of pre-cooling (Marino,

2002) and the lack of change in physiological state occurring from neck cooling collar (Tyler and Sunderland, 2011b) is not uncommon to report. To date, little is known on the benefits of pre- and per-cooling interventions in highly trained individuals as the majority of investigations have primarily included healthy/moderately trained participants.

The rationale for male participation was based upon the sex-related differences in body fat composition and rectal temperature responses due to the menstrual cycle that would interfere with the physiological and perceptual responses to the cooling intervention. The disadvantage of excluding female participation in this study does not allow for the conclusion to be made that the outcome of the study would be similar in female participants.

8.2.3. The effects of a short-term and medium-term 60 min daily isothermic heat acclimation protocol on the physiological, perceptual, cognitive function and endotoxin LPS response during heat stress

It was demonstrated that a 60 min daily isothermic HA protocol, where a target rectal temperature of ~38.5 °C was attained, provided a sufficient thermal impulse to elicit the physiological and perceptual adaptations. Five days (STHA) provided a sufficient cumulative thermal strain (9.85 ± 1.35 °C) to effectively lower physiological and perceptual strain; however, ten days (MTHA) elicited more beneficial adaptations to total sweat losses, final thermoregulatory strain, and perceptions of exertion, thermal strain

and comfort. An isothermic HA protocol had previously been investigated, however, exposure time per session had lasted for 90 min over the duration of the protocol. Therefore, investigating whether the attainment of the same target thermal strain (~38.5 °C) for a 30 min shorter duration per day (33.3% less) had not been previously explored. Similarly, to the author's knowledge there have been no other studies that have investigated an isothermic HA protocol on the effects on cognitive function or the endotoxin LPS response over a short- and medium-term time course. Currently, limited evidence exists on the effects of an acute exercise bout on cognitive function and whether an active HA protocol can offer any protection to cognitive function during heat stress. The data presented in Chapter 6 reported that an acute bout of exercise in the heat had no effect on reaction time or spatial working memory. An active short-term isothermic HA protocol had neither a positive or negative effect to cognitive function and medium-term isothermic HA protocol was found to slower response time at pre-exercise but improved working memory as task complexity increased. Data from Chapter 7 reported that an acute bout of exercise under heat stress did not result in an increase in circulating endotoxin LPS response. An isothermic HA protocol over a short- and medium-term time course did not elicit a sustained rise or within session increase in LPS response. Both sexes were included for participation in the present study but no sex-specific responses to HA were made. Females were required to record which phase of the menstrual cycle they were on and no female participate had taken oral contraception within three months of participation.

8.3. Practical applications of findings

The research conducted in this thesis was for the purpose of the applied application for practitioners and endurance athletes to integrate prior to and during competition performed in the heat. The three key interventions can be divided into acute (i.e. hydration and cooling) and chronic (i.e. heat acclimation) interventions and the use of all three should be considered, especially with upcoming sporting events scheduled to take place in known hot environmental conditions (i.e. Tokyo 2020 Olympic Games) and the increasing frequency of extreme heat waves.

The detrimental effects of hypohydration on exercise performance have been extensively investigated, with laboratory-controlled studies often reporting hypohydration (>2%) to negatively impact upon an individual's capacity and exercise performance (Cheuvront *et al.*, 2005; Logan-Sprenger *et al.*, 2015; James *et al.*, 2017; Funnell *et al.*, 2019). However, controversy exists, whereby during real-world competitions the fastest runners have the highest percentage of body mass losses and therefore are the most hypohydrated (Zouhal *et al.*, 2011). It is clear how hypohydration might impair exercise performance but the question that remained was to what extent does perceived hydration status have on an individual's ability to perform. Previous data has shown that deception can play a role on how an individual feels (i.e. increased perceived exertion) without physiological adjustments during deception of exercise duration (Eston *et al.*, 2012), environmental conditions and temperature of the body (Borg *et*

al., 2018). The data presented in this thesis reveal that perception and expectation did not influence 15 min cycling performance in healthy individuals. The question remains whether this response would occur in highly trained individuals, who potentially have a greater experience and knowledge of how hypohydration might influence exercise performance. Based upon these data and previous findings, practitioners may want to consider the detail of information communicated back to the athlete regarding the full extent of their hydration status. The author is aware that misleading an athlete could potentially have further implications in highly motivated individuals and further research is warranted. It is advised to not provide false information, as this may increase the risk of an athlete to overly exert themselves by overriding protective mechanisms in place to ensure excessive heat is not stored and exercise is completed within homeostatic limitations.

Other acute interventions that might be considered to help to combat the increased physiological and perceptual strain during exercise under heat stress are pre- and per-cooling interventions. The application of CWI provides a successful pre-cooling stimulus to lower an athlete's preliminary rectal temperature before the onset of exercise and therefore creating a greater margin to store more heat, and in theory, delaying heat induced decrements in exercise performance. Depending on the type of sport the athlete partakes in the application of per-cooling may be more challenging to incorporate. While data from per-cooling studies that have used ice jackets predominately show an improvement in exercise capacity

and performance (likely due to a larger surface area cooled), this method holds ecological limitations e.g. the mass of the jacket and the hobbling effect of a poor fit. With this in mind, and supported by data presented in this thesis, the author recommends that cooling the neck region may offer benefit when the athlete is exposed to less thermally stressful conditions (~30 ºC to 35 ºC) than conducted in the present study (>40 ºC). It is also advised that this should be replaced throughout exercise bout to ensure continued cooling stimulus is applied throughout competition, as shown in previous neck cooling collar research to improve exercise performance (Tyler, Wild and Sunderland, 2010; Tyler and Sunderland, 2011b).

The data presented in Chapter 5 show that an isothermic HA protocol is a successful intervention to induce adaptations to reduce physiological and perceptual strain during exercise under heat stress (Garrett *et al.*, 2014; Gibson *et al.*, 2015; Racinais *et al.*, 2015). These adaptations take place without the translocation of LPS into the portal circulation did not occur from an acute bout of exercise under heat stress or consecutive bouts, as identified from the data presented in Chapter 7. Therefore, there was no apparent risk of a HA isothermic protocol at inducing a systemic inflammatory response, which is known to suppress immune function, increasing central fatigue which potentially could impair exercise performance. Furthermore, a five (STHA) or ten day (MTHA) isothermic HA protocol was not overly exerting as an increase in circulating cortisol from an over-activation of the hypothalamic-pituitary-thyroid axis did not occur. While there are discrepancies within the literature on the effect of

acute heat stress on cognitive function, a high thermal strain has been shown to result in slower reaction times and impaired working memory. In an attempt to investigate this further, the data presented in Chapter 7 found that an isothermic HA protocol had neither a positive nor negative effect on reaction time response, while ten days of HA was effective at reducing the amount of errors made as task complexity increased. Considering all data presented from Chapters 5 to 7, it would be recommended that an athlete should undertake a minimum of five days of HA; however, a ten day HA protocol would offer greater protection to the athlete, as more complete adaptations occur to total sweat losses, final thermoregulatory strain, perceptions of exertion and thermal perceptions. Five days of a 60 min isothermic HA protocol would still provide a sufficient thermal strain to effectively lower physiological and perceptual strain during exercise heat stress.

The need to alleviate the increased physiological and perceptual strain associated with exercising under heat stress should be considered when planning for competition in order to help mitigate the detrimental effects of heat stress on performance. As with all interventions, the author advises that these interventions should be rehearsed during training to ensure barriers and logistical issues of interventions are overcome.

8.4. Future research

A number of further questions have arisen from the results obtained in the present body of work. The main questions are detailed below.

The effect of perception and expectation of hydration status on cycling performance in the heat in highly trained cyclists. Experience and knowledge of hydration status may be far greater in highly trained athletes compared to untrained populations and therefore, further research is warranted to investigate if the awareness of hydration status would be an influential factor on performance outcome in trained individuals.

The threshold rectal temperature for the use of pre- and per-cooling to improve prolonged exercise performance in highly trained individuals in extreme hot conditions. CWI and neck cooling collar have been demonstrated to be effective at improving exercise performance and capacity in the heat in other investigations; however, this benefit was not observed in the present study. This may be due to the strain being below a threshold for benefit and/or and it remains unclear what such a threshold is in elite and sub-elite athletes.

The effect of an isothermic running HA protocol on gastrointestinal permeability and circulating LPS and anti-LPS responses. In Chapter 7 of this thesis, a cycling isothermic HA protocol did not increase circulating LPS concentrations post-acute heat exposure and at any time point during the HA protocol. Intestinal permeability was not measured in this study and therefore, it is not possible to determine if heat exposure had an effect. Previous literature that used running as the mode of exercise found the LPS response to be elevated; however, uncertainty exists whether this response was due to exercise mode per se, thermal strain or

the combination of both. Further research is warranted to investigate whether a running protocol would elicit a similar response to previous running protocols or the findings in the current study.

8.5. Conclusion

The development of hyperthermia during exercise in the heat imposes a greater physiological and perceptual strain compared to exercise performed at the same relative exercise intensity in cooler environmental conditions, resulting in an impaired exercise capacity and performance. A number of interventions have been investigated to alleviate heat stress and to mitigate the detrimental effects associated with thermal strain, including; hydration, cooling and HA interventions. The data from the experimental chapters reported in this thesis highlight that for acute interventions awareness of hydration status in healthy individuals did not influence 15 min cycling TT performance. Furthermore, neither pre- nor per-cooling interventions improved 15 min cycling TT performance in highly trained individuals in the heat. For the chronic intervention, a daily 60 min isothermic HA protocol (target rectal temperature ~38.5 ºC) was successful at eliciting the necessary adaptations to reduce physiological and perceptual strain without the translocation of LPS or negative effects on cognitive function. Five days of HA provided a sufficient thermal strain to induce adaptations; however, ten days of HA offered a more complete adaptation.

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Appendices

Loughborough University		
Appendix A - Chapter 3	A1	Participant information sheet- front page
	A2	Consent form and health screening
	A3	Ethical application form - front page
University of Roehampton		
Appendix B- Chapter 4	B1	Participant information sheet – full copy
	B2	Consent form and health screening
	B3	Ethical application form - front page
Appendix C - Chapter 5-7	C1	Participant information sheet – front page
	C2	Consent form and health screening
	C3	Ethical application form - front page
Perceptual Scales & Calibration		
Appendix D	D1	Ratings of perceived exertion (RPE; Borg, 1982)
	D2	Thermal comfort (TC; Gagge, Stolwijk and Hardy, 1967)
	D3	Thermal sensation (TS; Young *et al.*, 1987)
Appendix E	E1	Calibration Measurements

Appendix A1

Front page of participant information sheet - Chapter 3

Loughborough University

Effect of carbohydrate and protein concentration of drinks ingested during exercise on exercise performance and post-exercise appetite.

Participant Information Sheet

What is the purpose of the study?
Numerous studies have examined the effect of manipulating the composition of drinks ingested during exercise, but many of these drink manipulations dramatically alter the taste profile of the ingested drink making it virtually impossible to truly blind volunteers to the drink contents. Most of these studies have examined different drink compositions ingested during exercise in a temperate environment and very little is known about how the different drinks influence exercise performance/ responses during exercise in warm environments.

This study will use a gastric feeding tube to deliver the drink directly to the stomach during exercise and therefore allow the effects of two different drink compositions to be examined in a completely blinded manner for the first time.

Who is doing this research and why?
Miss Jodie Moss is conducting this study as part of her PhD, and the study is being supervised by Dr Lewis James. This study is part of a student research project supported by Loughborough University.

Are there any exclusion criteria?
You must be aged between 18 and 45 years, a healthy moderately active male, a non-smoker and have no known history of gastric, digestive, cardiovascular or renal disease.

Once I take part, can I change my mind?
Yes! After you have read this information and asked any questions you may have we will ask you to complete an Informed Consent Form, however if at any time before, during or after the sessions you wish to withdraw from the study please just contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing.

Your data may be withdrawn from the research project unless: final results have been published; your data is no longer identifiable because of coding/ anonymity; or your data cannot be extracted from cohort analysis.

Will I be required to attend any sessions and where will these be?
Yes. All trials will all be held in the Clyde Williams building, Loughborough University.

How long will it take?
Three preliminary trials lasting ~1-4 h.
Two experimental trials lasting ~4 h.

Is there anything I need to do before the sessions?
You will need to adhere to certain dietary guidelines before some of the sessions. These will be explained to you by an experimenter. The night before the trial (10pm) you will consume an ingestible pill with water, full instructions on what to do will be given in person.

Is there anything I need to bring with me?
Clothing appropriate for cycling exercise e.g. shorts, t-shirt, socks, training shoes

Appendix A2

Loughborough University

Effect of carbohydrate and protein concentration of drinks ingested during exercise on exercise performance and on post-exercise appetite.

INFORMED CONSENT FORM
(to be completed after Participant Information Sheet has been read)

Taking Part	**Please initial box**
The purpose and details of this study have been explained to me. I understand that this study is designed to further scientific knowledge and that all procedures have been approved by the Loughborough University Ethics Approvals (Human Participants) Sub-Committee.	☐
I have read and understood the information sheet and this consent form.	☐
I have had an opportunity to ask questions about my participation.	☐
I understand that I am under no obligation to take part in the study, have the right to withdraw from this study at any stage for any reason, and will not be required to explain my reasons for withdrawing.	☐
I agree to take part in this study.	☐
Use of Information	
I understand that all the personal information I provide will be treated in strict confidence and will be kept anonymous and confidential to the researchers unless (under the statutory obligations of the agencies which the researchers are working with), it is judged that confidentiality will have to be breached for the safety of the participant or others or for audit by regulatory authorities.	☐
I agree to assign the copyright I hold in any materials related to this project to Dr Lewis James and Miss Jodie Moss.	☐
Bodily Samples	
I agree that the bodily samples taken during this study can **only be** used for this study and will be <u>disposed of within 3 years.</u>	☐

_____		_____	_____
Name of participant	[printed]	Signature	Date
_____		_____	_____
Researcher	[printed]	Signature	Date

Loughborough University

Name .....
Date of Birth .....

Health Screen Questionnaire for Study Volunteers

As a volunteer participating in a research study, it is important that you are currently in good health and have had no significant medical problems in the past. This is (i) to ensure your own continuing well-being and (ii) to avoid the possibility of individual health issues confounding study outcomes.

If you have a blood-borne virus, or think that you may have one, please do not take part in this research.

Please complete this brief questionnaire to confirm your fitness to participate:

1. **At present,** do you have any health problem for which you are:

(a)	on medication, prescribed or otherwise	Yes	☐	No	☐
(b)	attending your general practitioner.....	Yes	☐	No	☐
(c)	on a hospital waiting list	Yes	☐	No	☐

2. **In the past two years,** have you had any illness or injury which required you to:

(a)	consult your GP.....	Yes	☐	No	☐
(b)	attend a hospital outpatient department.....	Yes	☐	No	☐
(c)	be admitted to hospital	Yes	☐	No	☐

3. **Have you ever** had any of the following:

(a)	Convulsions/epilepsy	Yes	☐	No	☐
(b)	Asthma	Yes	☐	No	☐
(c)	Eczema	Yes	☐	No	☐
(d)	Diabetes	Yes	☐	No	☐
(e)	A blood disorder	Yes	☐	No	☐
(f)	Head injury	Yes	☐	No	☐
(g)	Digestive problems	Yes	☐	No	☐
(h)	Heart problems/chest pains	Yes	☐	No	☐
(i)	Problems with muscles, bones or joints	Yes	☐	No	☐
(j)	Disturbance of balance/coordination	Yes	☐	No	☐
(k)	Numbness in hands or feet	Yes	☐	No	☐
(l)	Disturbance of vision	Yes	☐	No	☐
(m)	Ear/hearing problems	Yes	☐	No	☐
(n)	Thyroid problems	Yes	☐	No	☐
(o)	Kidney or liver problems	Yes	☐	No	☐
(p)	Problems with blood pressure	Yes	☐	No	☐

If YES to any question, please describe briefly if you wish (eg to confirm problem was/is short-lived, insignificant or well controlled.)

.....

4. Smoking, physical activity and family history

(a)	Are you a current or recent (within the last six months) smoker?	Yes ☐	No ☐
(b)	Are you physically active (30 minutes of moderate intensity, physical activity on at least 3 days each week for at least 3 months)?	Yes ☐	No ☐
(c)	Has any, otherwise healthy, member of your family under the age of 35 died suddenly during or soon after exercise?	Yes ☐	No ☐

5. Allergy Information

(a)	Are you allergic to any food products?	Yes ☐	No ☐
(b)	Are you allergic to any medicines?	Yes ☐	No ☐
(c)	Are you allergic to plasters?	Yes ☐	No ☐
(d)	Are you allergic to latex?	Yes ☐	No ☐

If YES to any of the above, please provide additional information on the allergy

.....

6. Are you currently involved in any other research studies at the University or elsewhere?

Yes ☐ No ☐

If yes, please provide details.

.....

7. Please provide contact details of a suitable person for us to contact in the event of any incident or emergency.

Name:
.....

Telephone Number:
.....

Work ☐ Home ☐ Mobile ☐

Relationship to Participant:.....

Appendix A3

The research for this project was submitted for ethics consideration under the reference R15-P045 in the School of Sport, Exercise and Health Sciences and was approved under the procedures of Loughborough University's Ethics Committee on 01/03/2015.

Ethics Approvals (Human Participants) Sub-Committee

Loughborough University

Research Proposal for Studies Involving Human Participants

Project Details

1. Project Title: Effects of carbohydrate and protein intake ingested during exercise on exercise performance and post -exercise appetite.

2. Aims and objectives of the study

The primary aim of this study is to examine the effect of perception and expectation of hypohydration on exercise performance, whilst the secondary aim is to examine the effect of changes in hydration state on subsequent fluid and food intake and markers of appetite (hormones and subjective appetite).

NOTE: Given the nature of the study, it is important that the true purpose of the investigation is hidden from the participants until they have completed the experimental protocol. Therefore the background and aims provided in the participant information sheet does not match the study aims outlined here. Description of the study demands, however, is accurate.

3. Lay summary of the study
Note: This should be understandable to a non-expert and should not be a copy of the research proposal. It should include the reasons for the research, the background to it and why the area is important to investigate.

There have been many studies that have investigated the effect of dehydration (a loss of body water) on exercise performance and the general consensus is that a reduction in total body water during exercise equivalent to a loss of 2-3% body mass impairs aerobic exercise performance in temperate and warm environmental conditions. The design of all these studies is such that subjects have been provided either sufficient fluid to match fluid losses during exercise, or no/very little fluid, and as such know whether they are performing the hydrated/ dehydrated trial. The fact that not one study has blinded subjects to their hydration state during exercise is a major limitation in the interpretation of this research area, particularly since there is a strong public perception that dehydration is negative. This will be the first study to investigate exercise-induced dehydration in a blinded fashion and as such will provide vital information as to whether mild dehydration (~3%) has any effect on exercise performance.

4. Start date of study: 16/03/2015

Appendix B1

The Extreme Environments Laboratory
Sport and Exercise Sciences

Department of Life Sciences
University of Roehampton
Whitelands College
Holybourne Avenue
London, SW15 4JD

Tel: 020 8392 3536

The effect of different cooling strategies applied before and during cycling in the heat

What is the purpose of the study?
Numerous of studies have examined the effect of ambient temperature on exercise performance, however there is a limited number that have explored the endoxtoxemic response that occurs. Endotoxemia has also been referenced as "leaky gut" where endotoxins derived from gram negative bacteria is released from the gastrointestinal tract into the blood, causing symptoms including nausea, bloating and cramping. Most of these studies have reported only the physiological responses that occur (e.g. body core temperature & heart rate response), but very little is known about the which may influence exercise performance in cool and hot environmental conditions.

Who is doing this research and why?
Miss Jodie Moss is conducting this study as part of her PhD.

Are there any exclusion criteria?
You must be: aged between 18 and 50 years, a training cyclist/triathlete for >2 yrs, a non-smoker and have no known history of gastric, digestive, cardiovascular or renal disease.

Once I take part, can I change my mind?
Yes, after you have read this information and asked any questions you may have, we will ask you to complete an Informed Consent Form, however if at any time before, during or after the sessions you wish to withdraw from the study please contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing.

Your data may be withdrawn from the research project unless: final results have been published; your data is no longer identifiable because of coding/ anonymity; or cannot be extracted from cohort analysis.

How long will it take?
One preliminary trial combined with a full familiarisation of the experimental trial, will last approximately 3h to 3h 30 min.
Five experimental trials lasting 2 hr 30 min, will take place on separate occasions, 7 days apart.
All trials will take place in the physiology lab (B0035).

What should you wear?
Clothing appropriate for cycling exercise e.g. cycling shorts, t-shirt, socks and cycling shoes.

Is there anything I need to do before the sessions?
Please refrain from alcohol and caffeine 24 hrs before trial, and no strenuous exercise 48 hours before.

Appendix B2

University of Roehampton London

The Extreme Environments Laboratory
Sport and Exercise Sciences

Department of Life Sciences
University of Roehampton
Whitelands College
Holybourne Avenue
London, SW15 4JD

Tel: 020 8392 3536

Informed consent

	Initial
The purpose of this visit(s) has been clearly explained to me and any risks involved in my participation have been made clear	_____
I have completed the health screen accurately to the best of my knowledge	_____
All my questions about the visit(s) have been satisfactorily answered	_____
Optional: I am happy for my data to be used for research and publication purposes. I understand that the information I provide will be treated in confidence by the investigator and that my identity will be protected in the publication of any findings, and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University's Data Protection Policy.	_____

Name: _____

Signature: _____

Date: _____

Relevant Contact Details:
Name: Jodie Moss
Position: PhD Research Student
University address: University of Roehampton, Whiteland's College, Holybourne Avenue, London, SW15 4JD
Email: Mossj3@roehampton.ac.uk
Telephone: 0208 392 3536

Head of department contact details
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University address: 1068 Parkstead house, Whitelands
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Supervisor contact details
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Position: Senior Lecturer
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The Extreme Environments Laboratory
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Please assess your health status by marking all TRUE statements:

You have had:

_____ A heart attack
_____ Heart surgery
_____ Cardiac catheterisation
_____ Coronary angioplasty (PTCA)
_____ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
_____ Heart valve disease
_____ Heart failure
_____ Heart transplantation
_____ Congenital heart disease

Symptoms

_____ Chest discomfort with exertion
_____ Unreasonable breathlessness
_____ Dizziness, fainting or blackouts
_____ Take heart medication

Other health issues

_____ You have diabetes — Is it medically controlled? YES NO
_____ You have asthma — Is it medically controlled? YES NO
_____ You have burning sensation in your lower legs when walking short distances
_____ You have musculoskeletal problems that limit your physical activity
_____ You have concerns about the safety of exercise
_____ You take prescription medications — Please provide details:

_____ You are pregnant
_____ You are a man over 45 years of age
_____ You are a woman older than 55 years, have had a hysterectomy or are postmenopausal
_____ You smoke or quit within the last 6 months
_____ Your blood pressure is > 140/90 mm Hg
_____ You take blood pressure medication
_____ You have a close blood relative who had a heart attack before the age 55 (father or brother) or age 65 (mother or sister)
_____ You are physically inactive (less than 30 min at least 3 times a week)
_____ You are have a BMI over 25kg/m^2
_____ You suffer from epilepsy/convulsions

Modified from: AHA/ACSM Position Stand (1998), MSS, 30(6), 1009 – 1018

I confirm that the data above is accurate to the best of my knowledge as of the date below:

Name (PRINT) _____

Signature _____

Date _____

Appendix B3

The research for this project was submitted for ethics consideration under the reference LSC 17/208 in the Department of Life Sciences and was approved under the procedures of the University of Roehampton's Ethics Committee on 28/09/2017.

NAME: Jodie Moss **DEPARTMENT:** Life Sciences	**ETHICS REFERENCE:**

Roehampton University London

ETHICS

APPLICATION FORM (May 2017)

PLEASE CHECK THE RELEVANT BOX *(NB. double click on the check box and select 'checked')* MEMBER OF STAFF ☐ RESEARCH STUDENT ☒ (MPhil, PhD, EdD, PsychD) EXTERNAL INVESTIGATOR ☐ STUDENT (Other)** ☐ *If you are a transfer student or conducting collaborative research you may not need to complete this form, please see Section 2.2. of the Guidelines. **If you are on a taught course you do not need to complete this form unless your project is worth more than 50% of your total credits or you have been asked to do so by your supervisor*	
SECTION 1: PERSONAL DETAILS *Please complete the header with your name and Department*	
Name (lead):	Jodie Natasha Moss – Life Sciences
Other investigators:	Dr Christopher Tyler, Dr Richard Mackenzie, & Dr Steven Trangmar
Correspondence address:	Department of Life Sciences, Whitelands College, Holybourne Ave, London, SW15 4JD, UK.
Telephone no:	07831 653 653
Email:*(all correspondence will be sent by email unless otherwise requested)*	Mossj3@roehmapton.ac.uk
FOR STUDENTS ONLY:	
Programme of study & Department:	PhD. Life Sciences
Mode of study (full-time/part-time)	Full-time
Director of Studies: (If you are on a taught course please give the name of your supervisor)	Dr Christopher Tyler
FOR EXTERNAL INVESTIGATORS ONLY (please see Section 4.5 of the Ethical Guidelines):	
Name of Academic Assessor:	

1

Appendix C1

University of Roehampton London

The Extreme Environments Laboratory
Sport and Exercise Sciences

Department of Life Sciences
University of Roehampton
Whitelands College
Holybourne Avenue
London, SW15 4JD

Tel: 020 8392 3536

The effect of a short term and medium term heat acclimation on physiological adaptations to the heat

A brief overview of the study

The aim of this study is to capture the physiological adaptations that occur during a short term (5 day) and medium term (10 day) heat acclimation protocol in endurance trained athletes. Repeated exposure to heat with exercise produces acclimatization, where physiological changes occur to increase tolerance to heat stress. As it stands the current literature has highlighted the main physiological changes such as cardiovascular and thermoregulatory adaptations. However, there is limited data that shows the effects of short and medium term heat acclimation on the cellular and intestinal permeability responses.

Who is doing the research and why?

Miss Jodie Moss is conducting this study as part of her PhD, and the study is being supervised by Drs Tyler, Mackenzie, and Trangmar. This study is part of a student research projected supported by University of Roehampton.

Are there exclusion criteria?

You must between aged between 18 and 60 years, an endurance athlete for >2 yrs, a non-smoker and have no known history of gastric, digestive, cardiovascular or renal disease, this will be assessed on your first visit via a health screen questionnaire.

Once I take part, can I change my mind?

Yes, after you have read this information and asked any questions you may have, we will ask you to complete an Informed Consent Form, however if at any time before, during or after the sessions you wish to withdraw from the study please contact the main investigator. You can withdraw at any time, for any reason and you will not be asked to explain your reasons for withdrawing.

Your data may be withdrawn from the research project unless: results have been published; your data is no longer identifiable because of coding/ anonymity; or cannot be extracted from cohort analysis.

What should you wear/bring?

Clothing that is appropriate for exercise e.g. shorts, t-shirt, socks, trainers. If you wish to bring your rucksacks/hydration packs to test out, then you can during the heat acclimation days. During the heat stress test, we will provide you with water bottles.

Appendix C2

University of Roehampton London

The Extreme Environments Laboratory
Sport and Exercise Sciences

Department of Life Sciences
University of Roehampton
Whitelands College
Holybourne Avenue
London, SW15 4JD

Tel: 020 8392 3536

PARTICIPANT CONSENT FORM

Investigator Contact Details:

Name: Jodie Moss
Department Life Sciences
University Address: Whitelands College, Holybourne Avenue, London. SW15 4JD
Email: Mossj3@roehampton.ac.uk
Telephone: 0208 392 3536

Consent Statement:

I agree to take part in this research and am aware that I am free to withdraw at any point without giving a reason, although if I do so I understand that my data might still be used in a collated form. I understand that the information I provide will be treated in confidence by the investigator and that my identity will be protected in the publication of any findings, and that data will be collected and processed in accordance with the Data Protection Act 1998 and with the University's Data Protection Policy.

Name .....

Signature .....

Date .....

Please note: if you have a concern about any aspect of your participation or any other queries please raise this with the investigator (or if the researcher is a student you can also contact the Director of Studies.) However, if you would like to contact an independent party please contact the Head of Department.

Head of Department Contact Details:

Name: Dr Caroline Ross
University Address: Whitelands College, Holybourne Avenue, London, SW15 4JD.
Email: c.ross@roehampton.ac.uk
Telephone: 0208 392 3529

Director of Studies Contact Details:

Name: Dr Richard MacKenzie
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University of Roehampton London

The Extreme Environments Laboratory
Sport and Exercise Sciences

Department of Life Sciences
University of Roehampton
Whitelands College
Holybourne Avenue
London, SW15 4JD

Tel: 020 8392 3536

Please assess your health status by marking all TRUE statements:

You have had:

_____ A heart attack
_____ Heart surgery
_____ Cardiac catheterisation
_____ Coronary angioplasty (PTCA)
_____ Pacemaker/implantable cardiac defibrillator/rhythm disturbance
_____ Heart valve disease
_____ Heart failure
_____ Heart transplantation
_____ Congenital heart disease

Symptoms

_____ Chest discomfort with exertion
_____ Unreasonable breathlessness
_____ Dizziness, fainting or blackouts
_____ Take heart medication

Other health issues

_____ You have diabetes — Is it medically controlled? YES NO
_____ You have asthma — Is it medically controlled? YES NO
_____ You have burning sensation in your lower legs when walking short distances
_____ You have musculoskeletal problems that limit your physical activity
_____ You have concerns about the safety of exercise
_____ You take prescription medications — Please provide details:

_____ You are pregnant
_____ You are a man over 45 years of age
_____ You are a woman older than 55 years, have had a hysterectomy or are postmenopausal
_____ You smoke or quit within the last 6 months
_____ Your blood pressure is > 140/90 mm Hg
_____ You take blood pressure medication
_____ You have a close blood relative who had a heart attack before the age 55 (father or brother) or age 65 (mother or sister)
_____ You are physically inactive (less than 30 min at least 3 times a week)
_____ You are have a BMI over 25kg/m^2
_____ You suffer from epilepsy/convulsions

Modified from: AHA/ACSM Position Stand (1998), MSS, 30(6), 1009 – 1018

I confirm that the data above is accurate to the best of my knowledge as of the date below:

Name (PRINT) _____

Signature _____

Date _____

Appendix C3

The research for this project was submitted for ethics consideration under the reference LSC 18/228 in the Department of Life Sciences and was approved under the procedures of the University of Roehampton's Ethics Committee on 21/03/2018.

NAME: Jodie Moss
DEPARTMENT: Life Sciences

ETHICS REFERENCE:

University of Roehampton London

ETHICS APPLICATION FORM (Staff and Research Students)

Sept 2017

PLEASE CHECK THE RELEVANT BOX

(NB. double click on the check box and select 'checked')

MEMBER OF STAFF ☐ RESEARCH STUDENT ☒ (MPhil, PhD, EdD, PsychD)

EXTERNAL INVESTIGATOR ☐ STUDENT (Other)** ☐

*If you are a transfer student or conducting collaborative research you may not need to complete this form: please see Section 2.2. of the Guidelines. **If you are on a taught course you do not need to complete this form unless your project is worth more than 50% of your total credits or you have been asked to do so by your supervisor*

SECTION 1: PERSONAL DETAILS *Please complete the header with your name and Department*	
Name (lead):	Jodie Moss
Other investigators:	Dr Christopher Tyler Tom Reeve Dr Steven Trangmar Dr Richard Mackenzie
Correspondence address:	Department of Life Sciences, Whitelands College, Holybourne Ave, London SW15 4JD
Telephone no:	07831 653 653
Email: *(all correspondence will be sent by email unless otherwise requested)*	Mossj3@roehampton.ac.uk
FOR STUDENTS ONLY:	
Programme of Study & Department:	PhD. Life Science
Mode of study (full-time/part-time)	Full Time
Director of Studies & Supervisor: (If you are on a taught course please just give the name of your supervisor)	Director of Studies: Dr Richard Mackenzie Supervisors: Dr Chris Tyler, Dr Steve Trangmar
FOR EXTERNAL INVESTIGATORS ONLY (please see Section 4.5 of the Ethical Guidelines):	
Name of Academic Assessor:	

1

Appendix D1

Rating of Perceived Exertion Scale (RPE) (Borg, 1982)

Rating of Perceived Exertion	
Rating	Perception of effort
6	No effort
7	Very, very light
8	
9	Very light
10	
11	
12	
13	Somewhat hard
14	
15	Hard
16	
17	Very hard
18	
19	Very, very hard
20	Maximal effort

Appendix D2

Thermal Comfort (TC) (Gagge, Stolwijk and Hardy, 1967)

Thermal Comfort	
Rating	Descriptive Information
1	Comfortable
2	Slightly Uncomfortable
3	Uncomfortable
4	Very Uncomfortable

Appendix D3

Thermal Sensation (TS) (Young *et al.,* 1987)

Thermal Sensation	
Rating	Descriptive Information
1	Cold
2	Cool
3	Slightly Cool
4	Neutral
5	Slightly Warm
6	Warm
7	Hot

Appendix E1 - Calibration Measurements

Oxygen uptake measurements

Metabolic online gas analysis – Chapters 4, 5, 6 and 7

During the preliminary trial expired metabolic gas was measured using an online metabolic cart (Oxycon Pro Jaeger, Germany) calibrated prior to the test using the flow from a 3 L syringe through a turbine for volume calibration and a two-point calibration using air and gases of known concentrations for O_2 (15.13%) and CO_2 (5.04%) fractions. The Oxycon Pro has been previously shown to be a valid and reliable system to use, with a low CV of <7% (Carter and Jeukendrup, 2002).

Douglas bag – Chapters 3 and 5

Prior to use, the Douglas bags were emptied, and the gas analyser was calibrated using room air and gas of known concentrations for O_2 (15.13%) and CO_2 (5.04%).

Hydration Measurements

Urine osmolality – Chapter 3

Daily calibration with distilled water was placed onto the osmometer before pressing the zero button. Following calibration, a small volume (~ 50 µL) of urine was secreted into the osmometer and start button was pressed to determine osmotic strength.

Urine specific gravity (USG) – Chapters 4, 5, 6 and 7

Daily calibration with distilled water (USG = 1.000) was performed prior to urine sampling. Distilled water was placed into a glass beaker and the refractometer pen was held downwards before pressing the zero key.

Thermoregulatory Measurements

Rectal temperature – Chapters 4, 5, 6 and 7

Before the start of each experimental study rectal thermistors were calibrated using a water-bath immersion and measured against a mercury thermometer. Accuracy of each rectal thermistor was assessed over 1 ºC intervals starting at 35 ºC to 41 ºC. During each experimental study participant used the same, sterilised, rectal thermistor for each trial. Rectal thermistors were used in the experimental studies completed at the University of Roehampton due to equipment availability.

Gastrointestinal temperature – Chapter 3

Due to equipment availability at Loughborough University this was the method chosen. Before a telemetry pill was given to the participant the temperature capsule were calibrated by using the data receiver in accordance with manufacturer instructions.

Skin temperature – Chapters 4, 5, 6 and 7

Prior to participant arrival, each iButton was activated following manufacturer's instructions. The iButtons were placed on the participant on the same location throughout experimental trial and by the same researcher. Furthermore, the same

ibutton was kept on the location area allocated on the participant throughout experimental trial.

Body fat percentage (%)

BodPod – Chapter 5

The BodPod was calibrated before use using a standard 2-point calibration process using the electronic weighing scales, calibrated weights and calibration cylinder (~50 L) and following the software instructions. Participants wore a fitted swimwear/shorts and a swim hat to cover all hair.